University of Alberta

BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF 5HT-RELATED DRUGS IN A MODEL OF MESOLIMBIC DOPAMINE HYPERACTIVITY

by

SARAH LOUISE WADDOCK

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE

in

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ABSTRACT

The behavioral and neurochemical effects of serotonin (5-hydroxytryptamine;5HT) receptor-related ligands on the activity of the mesolimbic dopamine (DA) system were investigated using a nicotine-induced locomotor hyperactivity model. 8-OHDPAT increased the locomotor activity of saline-treated rats and NAN 190 decreased the locomotor activity of nicotine-treated rats. Individually, ritanserin, (±)-pindolol and WAY 100635 did not alter behavior. TFMPP decreased locomotor activity but RU 24969 had opposite effects in both saline- and nicotine-treated rats. Effects of RU 24969 were blocked by (±)-pindolol but the effects of TFMPP were not blocked by (±)-pindolol or WAY 100635. Nicotine, 8-OHDPAT and TFMPP all increased hippocampal DA levels, whereas NAN 190 decreased hippocampal DA. These results demonstrate that significant differences exist between the actions of compounds classified as serotonin $5HT_{1A}$ and the $5HT_{1B}$ receptor agonists, respectively, and indicates that the current classification scheme for these compounds needs to be refined.

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ABBREVIATIONS

5-HIAA	5-hydroxyindoleacetic acid
5HT	5-hydroxytryptamine, serotonin
8-OHDPAT	8-hydroxy-2-(di-*n*-propylamino)tetralin
CNuc	caudate nucleus
d	day(s)
DA	dopamine
DOPAC	3,4-dihydroxyphenylacetic acid
DRN	dorsal raphé nucleus
g	gram(s)
GABA	γ-aminobutyric acid
h	hour(s)
HPLC-ED	high performance liquid chromatography with electrochemical detection
HVA	homovanillic acid
ip	intraperitoneal
iv	intravenous
kg	kilogram(s) (10^3 gram)
l	litre(s)
MAO	monoamine oxidase
mg	milligram(s)
min	minute(s)
ml	millilitre(s)
mM	millimolar (10^{-3} molar)
mPFC	medial prefrontal cortex

MRN	median raphé nucleus
NAcc	nucleus accumbens
NAN 190	1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl] piperazine
ng	nanogram(s) (10^{-9} gram)
nM	nanomolar (10^{-9} molar)
RU 24969	5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole
sc	subcutaneous
SEM	standard error of the mean
SN	substantia nigra
TFMPP	4-(3-trifluoromethylphenyl)piperazine
VTA	ventral tegmental area
wk	week(s)
μl	microlitre(s) (10^{-6} litre)
μM	micromolar (10^{-6} molar)

INTRODUCTION

A. GENERAL INTRODUCTION

The advent of modern psychiatric drug therapy can be accredited mainly to chlorpromazine, based on its successful treatment of mental disorders in 1952 [Clark and Del Guidice, 1970]. Subsequent studies on the therapeutic effects of psychopharmacological agents have provided evidence for the neurochemical basis of psychiatric diseases, and revealed the potential for rational drug development. In the development of pharmacological treatments for schizophrenia, for example, studies have shown that the catecholamine and serotonin systems are among the neural targets of effective drugs [Minchin and Csernansky, 1996; Meltzer and Maes, 1996].

To induce an effect, neurotransmitters must interact with receptors on neuronal membranes. Once released from the neuron, a neurotransmitter elicits an effect by binding to a receptor in a structure-dependent manner. In this way, each neurotransmitter binds to a distinct subset of receptors whose location in the brain will dictate the chemical's effects. The receptors may be located at either presynaptic or postsynaptic sites and can mediate a range of

responses. Many studies have suggested that not only abnormal levels of neurotransmitters, but also abnormal receptor populations, are likely causes of the psychiatric disorders [Joyce, 1993].

Unfortunately, efforts to clarify the biological basis of mental disorders has been hindered by many factors. Primarily, the ongoing discovery of novel neurotransmitters, neuromodulators and their receptors and the complex interactions of known neurotransmitter systems has made the development of a comprehensive portrait of the brain increasingly complex. Furthermore, the animal models used for certain research purposes may not be congruent with human application, and thus the results obtained may not be universally valid. Finally, the recent identification of the numerous receptors involved in neurotansmission has further expanded the knowledge base of psychiatric conditions.

Research efforts to date have paid considerable attention to individual neurotransmitter systems and their role in psychiatry. Specifically, interest has been focused on the role of monoamines, such as dopamine (DA) or serotonin (5-hydroxytryptamine; 5HT), in the pathology of these disorders.

Of course, these systems do not function independently *in vivo*. As the knowledge base in this area has increased, more studies have focused on neurotransmitter interactions, and on the multiple effects induced by the administration of psychoactive drugs on these neurotransmitter systems. For example, a number of single neurotransmitter theories have been proposed for schizophrenia, including insufficient dopamine transmission in the frontal cortex, excessive noradrenaline or DA transmission in limbic areas, or abnormal GABA regulation [Brunello et al., 1995; Sachar, 1985]. The greatest therapeutic success, however, has been achieved with atypical antipsychotics, such as clozapine and risperidone, which alter both the dopamine and serotonergic systems [Kapur and Remington, 1996]. This observation suggests that studying the interactions between neurotransmitters may help to explain the underlying cause of disorders such as schizophrenia.

The proposal for this thesis was to study these interactions by investigating the role of 5HT receptors in regulating the activity of the forebrain dopaminergic pathways, specifically the mesolimbic system. Increased activation of this mesolimbic DA system has been implicated in the etiology of schizophrenia and this tract has also been identified as a target of many

antipsychotic drugs [Sachar,1985]. Accordingly, studying the DA-5HT interactions may increase the understanding of regulatory mechanisms of the mesolimbic system and will also be valuable in determining the mechanism of action of antipsychotic drugs. The mesolimbic DA pathway, extending from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc), is one site believed to be involved in the expression of locomotor behavior [Clarke et al., 1988]. Experimentally, the mesolimbic pathway becomes activated following repeated intermittent nicotine administration, resulting in increased DA release in the NAcc and locomotor hyperactivity in rats [Clarke and Kumar, 1983b]. In the experiments described in this thesis, 5HT receptor-related ligands were used to investigate the role of selected 5HT receptors in mesolimbic DA activity. In a novel approach, these compounds were tested in rats using a nicotine hyperactivity model, and both behavioral and neurochemical parameters were measured.

To gain a thorough understanding of the potential interactions between the DA and 5HT, it is necessary to describe the individual neurotransmitter systems. The following sections review the DA and 5HT neural systems.

B. DOPAMINE (DA)

B.1. DA Biochemistry, Metabolism and Anatomy

DA is a catecholamine derived from the amino acid, p-tyrosine. p-Tyrosine is hydroxylated by tyrosine hydroxylase to 3,4-dihydroxyphenylalanine (L-DOPA), which is decarboxylated by DOPA decarboxylase to yield DA. DA is metabolised by two enzymes, catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO), to its metabolites: 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) (Figure 1) [McIlwain and Bachelard, 1971].

The DA system originates from a central core of neurons in the midbrain (Figure 2). Within this core, the cell bodies of these neurons are further subdivided into groups in three distinct areas: (VTA), the substantia nigra (SN) and the retrorubral nucleus, also known as areas A10, A9 and A8 respectively [Jacobwitz, 1978]. The DA cell bodies of the VTA give rise to the mesocorticolimbic DA system by projections to the striatum (NAcc), limbic areas (hippocampus, amygdala) and the cortex. The DA cell bodies of the SN project mainly to the dorsal striatum (CNuc), forming the nigrostriatal

Figure 1: Dopamine Synthesis and Catabolism

[adapted from McIlwain and Bachelard, 1971]

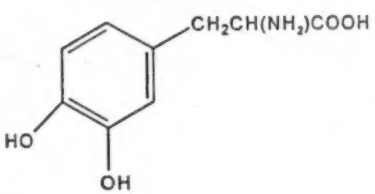
Synthesis

Phenylalanine

Phenylalanine hydroxylase

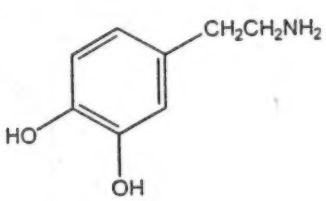
p-Tyrosine

Tyrosine hydroxylase



L-Dopa

L-Dopa decarboxyase

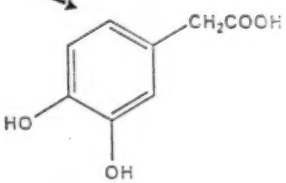


Dopamine

Catabolism

Dopamine

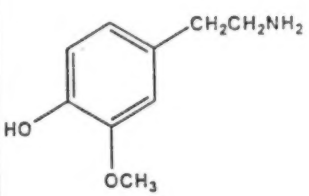
MAO + Aldehyde dehydrogenase



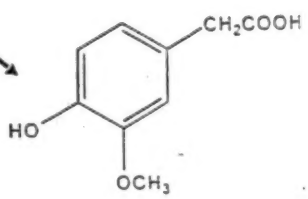
DOPAC

COMT

COMT



MAO + Aldehyde dehydrogenase



HVA (homovanillic acid)

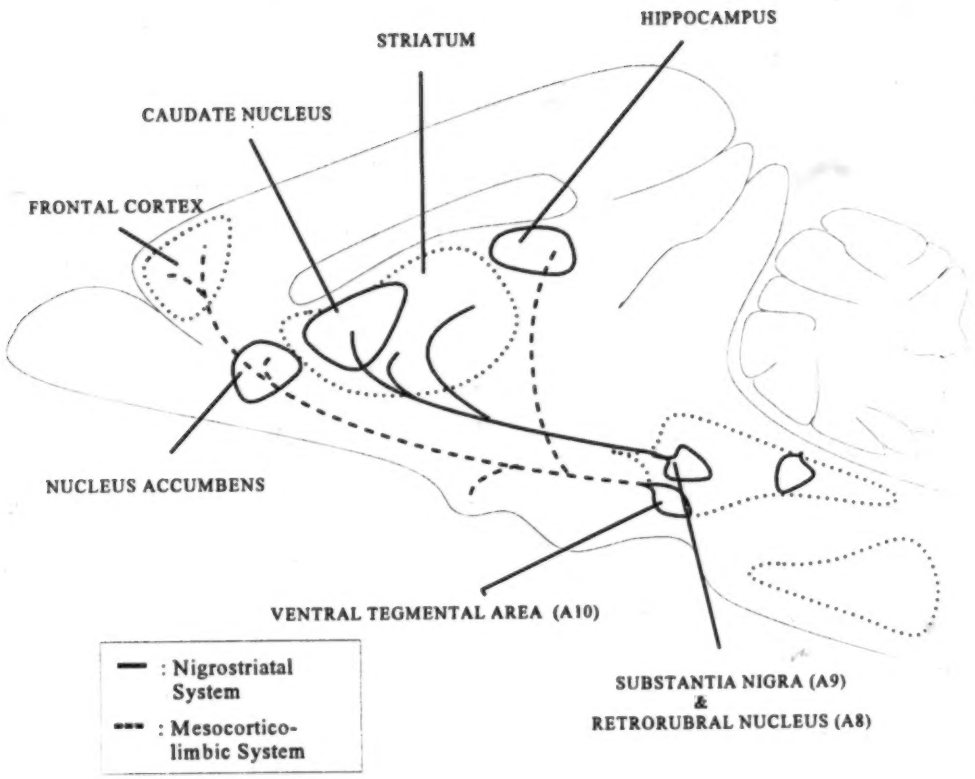


Figure 2: Principal Dopaminergic Systems in the Rat Brain

[adapted from Kelly, 1985]

system, an important part of the extrapyramidal motor system [Jacobowitz, 1978]. Both of these systems are responsible for regulating motor activity; the mesocorticolimbic system also coordinates motivation and reward [Kalivas, 1993]. In particular, the DA projections from the VTA to the NAcc mediate the locomotor activating effects recorded following administration of nicotine and many other psychomotor drugs [Clarke et al., 1988]. Functional abnormalities in both the mesolimbic and nigrostriatal DA circuits have been implicated in the etiology of psychiatric disorders, such as schizophrenia [Joyce, 1993].

B.2. DA Receptors

There are three general types of receptors, differentiated by their location relative to the primary neuron (Figure 3) [Boess and Martin, 1994; Martin and Humphrey, 1994; Wilcox and Gonzales, 1994]. Autoreceptors are found presynaptically on cell bodies (somatodendritic) or axons (terminal) and provide feedback on the extracellular concentration of the primary neurotransmitter. Postsynaptic receptors are located on adjacent neurons and mediate the postsynaptic effects of the primary neurotransmitter. Heteroreceptors are at presynaptic sites on secondary neurons and regulate

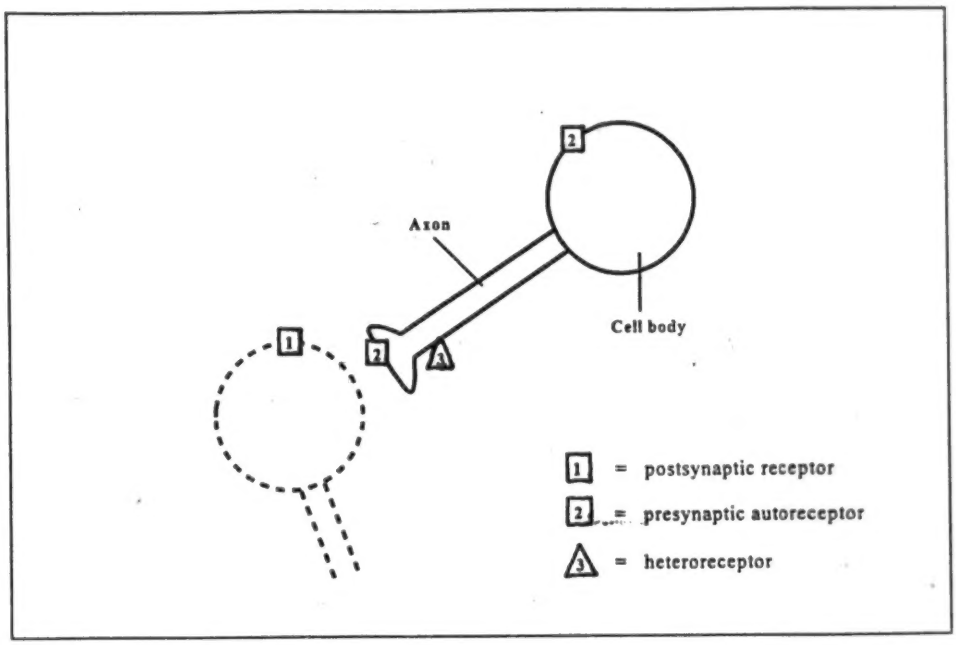


Figure 3: General Receptor Types

the activity of that neuron.

There are two families of dopamine receptors, namely the D_1-like DA receptor and D_2-like DA receptor families. The D_1-like DA family includes the D_1 and D_5 DA receptor subtypes, which are positively coupled to adenylyl cyclase and function at postsynaptic and terminal presynaptic sites [Civelli et al., 1993]. The D_2-like DA family includes the D_2, D_3, and D_4 DA receptor subtypes which function as autoreceptors at the dopamine cell bodies and as postsynaptic receptors, and are negatively coupled to adenylyl cyclase [Civelli et al., 1993; Kalivas, 1993]. Receptor binding studies show that D_1 and D_2 DA receptors are present in high concentrations in the cortex and striatum, respectively, whereas the D_3 and D_4 DA receptor subtypes are more prominent in limbic regions [Civelli et al., 1993; Kapur and Remington, 1996]. D_5 DA receptors are limited to the hippocampus and thalamic nuclei. Significantly, D_1 DA receptors have been identified in the VTA, SN or hippocampus; as have D_2 DA receptors [Cameron and Williams, 1995; Vezina, 1996]. The D_4 DA receptor has been intensely studied since it was identified as a potential target for the atypical antipsychotic, clozapine [Brunello et al., 1995; Civelli et al., 1993]. Its distribution is mainly in the

corticolimbic regions, and it has particularly low concentrations in striatal regions [Civelli et al., 1993].

There have been numerous functional studies on the DA receptors which demonstrate their varied effects. *In vitro*, stimulation of the midbrain somatodendritic D_2 and D_3 DA autoreceptors results in a decreased neuronal activity and a concurrent decreased DA release from the VTA or SN cell bodies and terminals [Civelli et al., 1993]. The stimulation of some D_2 DA receptor populations causes a decreased DA synthesis since some are linked to feedback mechanisms and others are not [Civelli et al., 1993]. Also, D_2 DA receptor antagonists have a hyperdopaminergic effect by releasing feedback inhibition of DA cell firing [Kalivas, 1993]. Stimulation of D_1 DA receptors appears to have no direct effect on the activity of DA neurons or their release of DA, although there is evidence of an indirect modulation of VTA neurons *via* GABA interneurons [Cameron and Williams, 1995]. It has been suggested that D_1 DA receptors regulate the release of other neurotransmitters, such as GABA and acetylcholine, from afferents which synapse on DA terminals in the striatum or cortex [Consolo et al., 1996; Kalivas, 1993]. D_4 and D_5 DA receptor effects are poorly understood at this

time [Civelli et al., 1993; Meador-Woodruff et al., 1992; Roth and Tandra, 1995]. Generally, the DA receptors play an important role in regulating the DA tone throughout the brain, *via* feedback inhibition and other regulatory mechanisms. This receptor-mediated level of DA activity, in turn, effects the terminal regions of the DA system, which are believed to mediate many different behavioral effects including locomotor activity and motivation.

B.3. DA and Schizophrenia

Based on clinical and postmortem studies, there are two opposite and regionally-selective dopaminergic theories of schizophrenia: patients exhibit either decreased dopaminergic activity in the frontal cortex, or excessive dopaminergic activity in the limbic and striatal regions [Sachar, 1985]. The role of DA in schizophrenia and antipsychotic mechanisms has been exhaustively reviewed and accordingly, the following is only a brief review [Brunello et al., 1995; Davis et al., 1991; Joyce, 1993; Kerwin and Taylor, 1996; Meltzer, 1996; Owens and Risch, 1995; Seeman, 1995]. In clinical studies, the traditional antipsychotics appear to act mainly by D_2 DA receptor blockade in the mesolimbic system. Chronic administration of these drugs leads to a depolarization blockade of the VTA neurons and, hence, a

decreased DA release from these neurons at the striatum and limbic areas [Meltzer, 1996]. Unfortunately, although the typical antipsychotics do treat the positive symptoms of schizophrenia, they also induce extrapyramidal parkinson-like symptoms (EPS), perhaps due to interactions with other neurotransmitter systems, such as 5HT [Kerwin and Taylor, 1996]. In contrast, the more effective atypical antipsychotics demonstrate significantly less D_2 DA receptor blockade, treat the positive and negative symptoms, and do not induce EPS in schizophrenic patients [Meltzer, 1996]. As mentioned above, the atypical antipsychotic clozapine preferentially binds to the D_4 DA receptor, and has a much lower affinity for D_1 and D_2 DA receptors [Brunello et al., 1995]. Currently, the primary focus of schizophrenia research is the interaction of DA with other neurochemical systems, including 5HT and glutamate.

C. SEROTONIN (5HT)

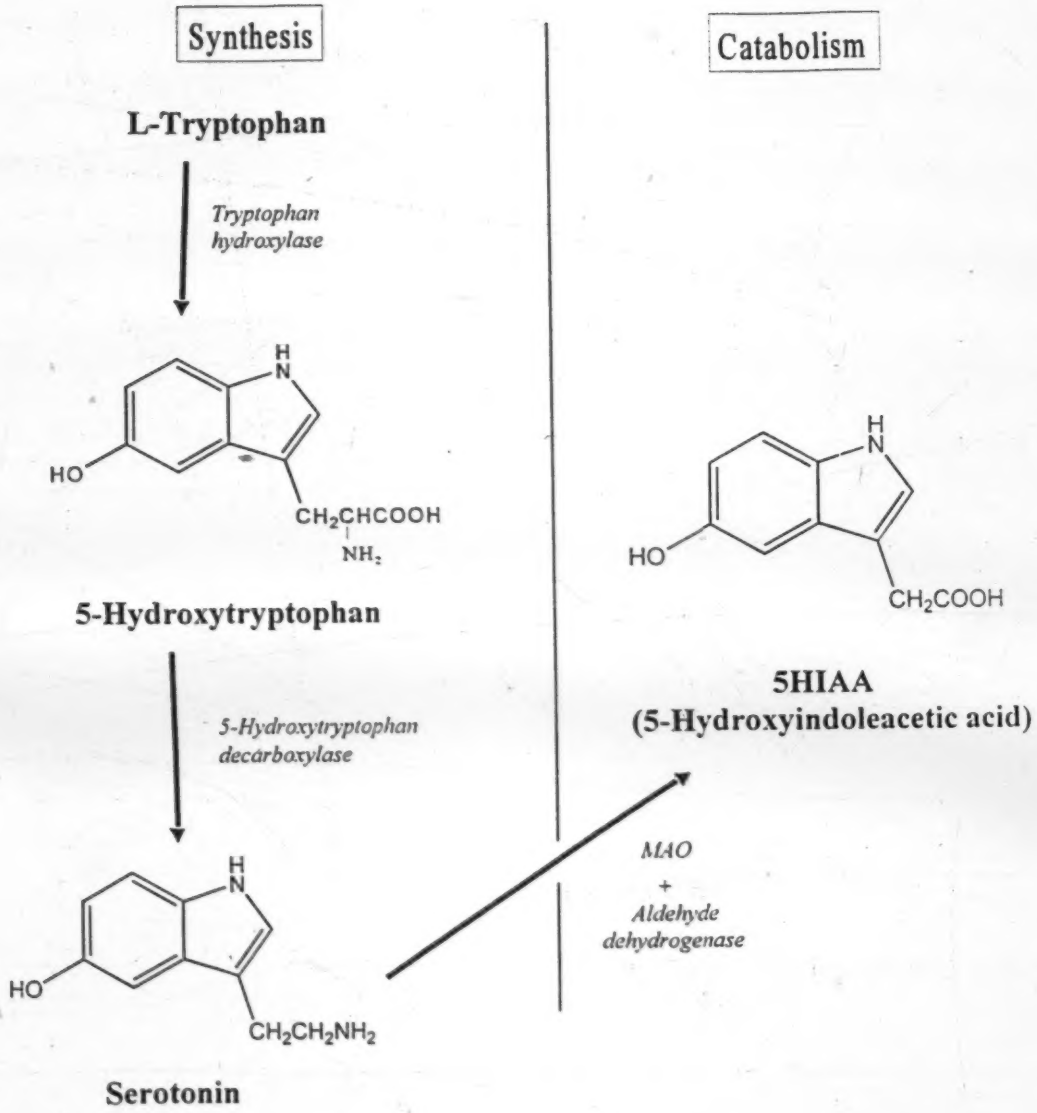
C.1. 5HT Biochemistry, Metabolism and Anatomy

Serotonin (5-hydroxytryptamine; 5HT) is a biogenic amine derived from the amino acid L-tryptophan. It is synthesized by the hydroxylation of tryptophan to 5-hydroxytryptophan, which is then decarboxylated to yield 5HT. Serotonin is metabolized by MAO and aldehyde dehydrogenase to its primary metabolite, 5-hydroxyindole acetic acid (5-HIAA) (Figure 4).

In the brain, the diffuse serotonergic system is derived from the raphé nuclei in the midbrain (Figure 5). The dorsal (DRN; area B7) and median (MRN; area B8) raphé nuclei have ascending projections to the brain, and more caudal raphé nuclei extend to sites in the periphery [Jacobowitz, 1978]. 5HT has a widespread innervation of the brain and, despite considerable overlap between the projections of the midbrain raphé nuclei, the DRN provides the primary serotonergic innervation of the basal ganglia, substantia nigra, striatum and amygdala, and the MRN preferentially projects to the medial septum [Kreiss and Lucki, 1994; Molliver, 1987]. Both nuclei innervate the hippocampus [Gobert et al., 1995]. Serotonin has been proposed to play a

Figure 4: Primary Routes of Serotonin and Synthesis and Catabolism

[adapted from McIlwain and Bachelard, 1971]


Synthesis
Catabolism
L-Tryptophan
Tryptophan hydroxylase
HO
H
N
CH2CHCOOH
NH2
5-Hydroxytryptophan
5-Hydroxytryptophan decarboxylase
HO
H
N
CH2CH2NH2
Serotonin
MAO
+
Aldehyde dehydrogenase
HO
H
N
CH2COOH
5HIAA
(5-Hydroxyindoleacetic acid)

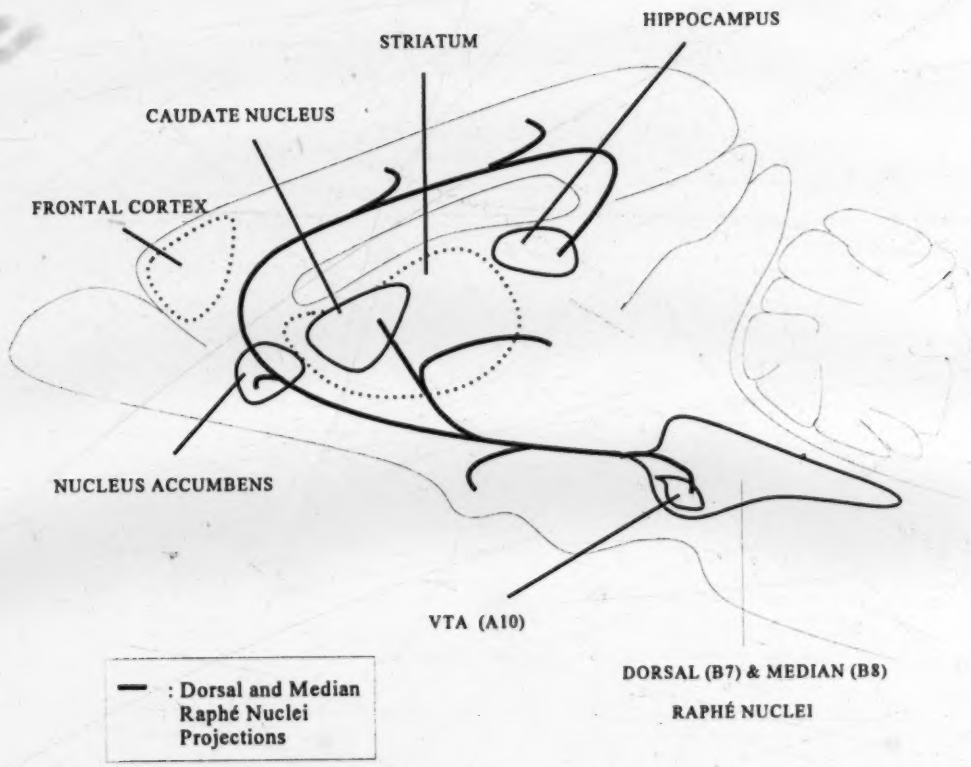


Figure 5: Serotonergic System in the Rat Brain

[adapted from Kelly, 1985]

role in the regulation of sleep, emotion, nociception, feeding and locomotor activity [Saito et al., 1996].

C.2. 5HT Receptors

The classification of serotonergic receptors has been repeatedly revised in recent years due to the identification of multiple novel receptor types and subtypes. Originally, three classes were recognized, namely $5HT_1$-like, $5HT_2$ and $5HT_3$ [Middlemiss and Tricklebank, 1992]. The recent convention includes 7 distinctive 5HT receptor classes based on transductional, structural and operational characteristics [Boess and Martin, 1994; Martin and Humphrey, 1994]. This classification, which includes information related to the receptor structure, distribution and linkage to transduction mechanisms, is shown in Table 1. The structure and intracellular mechanisms of these receptors have been reviewed elsewhere and therefore, are not described in this thesis [Boess and Martin, 1994; Martin and Humphrey, 1994]. In the current classification, the $5HT_{1B}$ and $5HT_{1D\beta}$ receptors are recognized as having a high degree of homology and sharing a similar distribution, but they are species-specific: the $5HT_{1B}$ subtype is found mainly in mice and rats of the species studied to date [Launay et al., 1994]. In addition, since the $5HT_{1C}$

Table 1: 5HT Receptor Classification (1996)

Class	Structure	Transduction	Subtypes	Distribution
$5HT_1$	7 TM, no introns	GPC, mainly inhibit AC	1A	A,OT,H,T, RN,C,L
			1B	SN,OT,CN, RN,NA,C
			1D (α, β)	OT,CN,NA, H,RN,C
			1E	C,CN,SN,H
			1 F(1Eβ)	C,S,H,T,RN
$5HT_2$	7 TM, introns and exons	GPC, IP_3 production	2A	C,CN,H,NA,S
			2B	C,CN,H,NA,S
			2C (1C)	L,BG,H,CP, H, SN, OT,C,RN
$5HT_3$	Ion channel	Conducts Na^+, K^+, Ca^+		C,A,H,OT,S, NA
$5HT_4$	7 TM	GPC, activate AC		H,SN,OT,S, NA
$5HT_5$	7 TM	not GPC, unknown	5A	H,C,T,S,A
			5B	H,RN,C
$5HT_6$	7 TM	GPC, activate AC		S,OT,H,C
$5HT_7$	7 TM	GPC, activate AC		T,H,L,C,S, OT,RN

A=amygdala, AC=adenylyl cyclase, BG=basal ganglia, C=cortex, CN=caudate nucleus, CP=choroid plexus, GPC=G protein coupled, H=hippocampus, IP_3 = inositol phosphate, NA=nucleus accumbens, OT=olfactory tubercle, RN=raphe nuclei, S=striatum, SN=substantia nigra, T=thalamic nuclei, TM = transmembrane regions

[data from Bruinvels et al., 1994; Brunivels et al., 1993a and 1993b; Boess and Martin, 1994; Martin and Humphrey, 1994 and Saito et al., 1996]

receptor was found to have an identical linkage to second messenger systems as the $5HT_{2C}$ receptor, the two subtypes were combined and renamed accordingly [Boess and Martin, 1994].

The 5HT receptors can be found at postsynaptic sites or presynaptically on non- 5HT-neurons (heteroreceptors) [Boess and Martin, 1994; Martin and Humphrey, 1994]. In addition, two of the receptors have been characterized as presynaptic autoreceptors: $5HT_{1A}$ at somatodendritic sites, and $5HT_{1B}$ at terminal sites (Figure 6). The regional distribution of the 5HT receptors throughout the brain is heterogeneous as shown in Table 1.

The functions associated with each of these 5HT receptors have not yet been clearly elucidated. This delay is due primarily to the limited number of selective ligands available; a summary of the most current receptor-specific compounds is shown in Table 2. A large body of research supports the role of the $5HT_{1A}$ receptor as a somatodendritic autoreceptor in the DRN and MRN, as a postsynaptic receptor in limbic areas and as a presynaptic heteroreceptor on DA terminals. *In vivo* and *in vitro* techniques have demonstrated a decrease in DRN cell firing activity following stimulation of

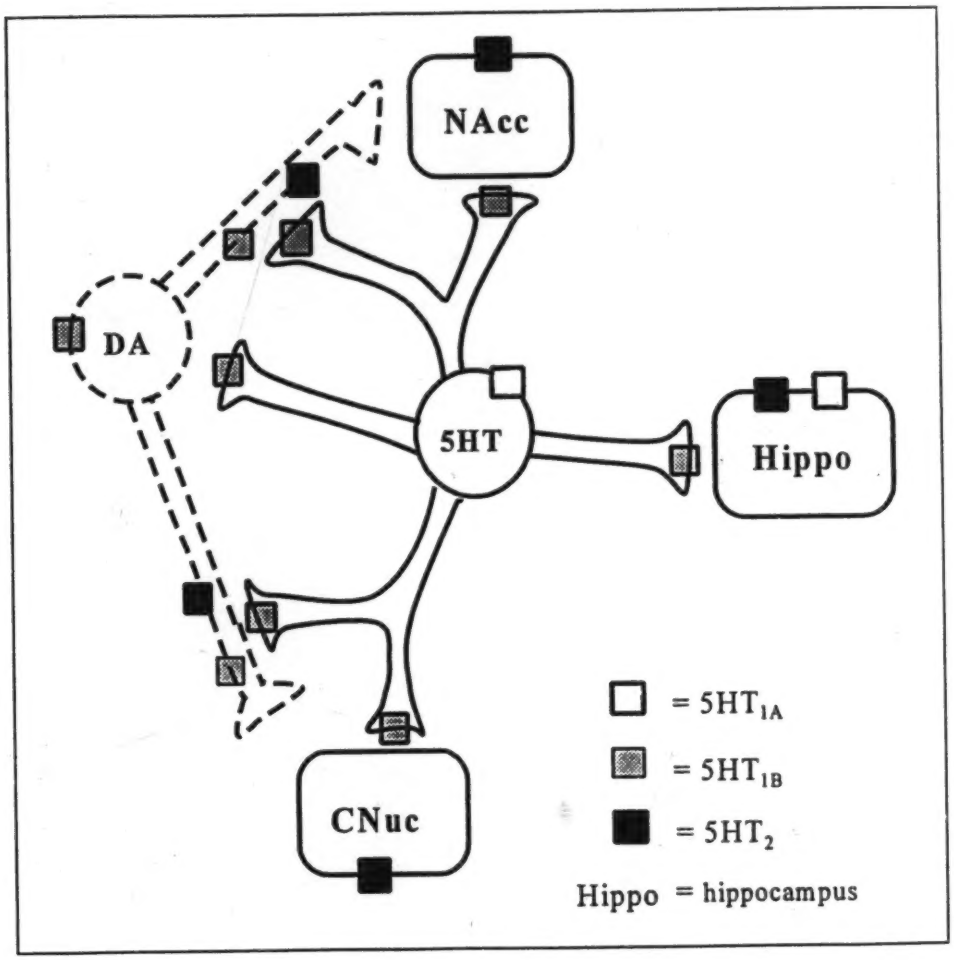


Figure 6: Selected 5HT Receptor Sites in the Rat Brain

[adapted from Kapur and Remington, 1996; Middlemiss and Hutson, 1994]

these receptors [Fletcher et al., 1995; Saito et al., 1996]. In addition, 5HT synthesis, turnover, and release from the DRN and terminal sites are also decreased [Gobert et al., 1995; Matos et al., 1996; Nomikos et al., 1992; Saito et al., 1996]. This autoreceptor stimulation has been effectively blocked by administration of the selective $5HT_{1A}$ receptor antagonist, WAY

Table 2: Selective 5HT Receptor Ligands

Receptor	Agonist	Antagonist
$5HT_{1A}$	8-OHDPAT	WAY 100635
$5HT_{1B}$	CP3129	
$5HT_{1D\alpha}$	sumatriptan	
$5HT_{1D\beta}$	sumatriptan	
$5HT_{1E}$		
$5HT_{1F}$		
$5HT_{2A}$	α-Me-5-HT	ketanserin
$5HT_{2B}$	α-Me-5-HT	LY53857
$5HT_{2C}$	RO-60-0175, RO-60-0332	mesulergine
$5HT_3$	2-Me-5-HT	odansetron
$5HT_4$	5-MT	GR113808
$5HT_{5A}$		
$5HT_{5B}$		
$5HT_6$		
$5HT_7$		

[Launay et al., 1994; Moreau et al., 1996]

100635 [Fletcher et al., 1995]. Specifically, the terminal sites affected by $5HT_{1A}$ receptor agonists are in the striatum and hippocampus [Saito et al., 1996]. *In vivo* studies demonstrate similar changes with systemic or intra-DRN $5HT_{1A}$ receptor agonist administration[Saito et al., 1996]. The existence of tonic inhibitory control of the DRN and MRN cells by $5HT_{1A}$ receptors is controversial: blockade of the autoreceptors has been reported to either have no effect [Gobert et al., 1995; Saito et al., 1996] or to increase 5HT release [Matos et al., 1996; Mundey et al., 1996]. Stimulation of $5HT_{1A}$ receptors in the DRN is believed to produce the distinctive behavioral effect, the "serotonergic syndrome", which is blocked by $5HT_{1A}$ receptor antagonists [Ahlenius and Salmi, 1995; Fletcher et al., 1995; Goodwin and Green, 1985; Mundey et al., 1996; Nomikos et al., 1992]. In rats, this syndrome includes an increased forward locomotion, suppressed rearing activity, forepaw treading, flat-body posture and straub tail [Jacobs, 1991]. However, early studies demonstrate that midbrain transection does not eliminate these behaviors which suggest that the syndrome may be primarily mediated at the level of the brainstem [Jacobs, 1976].

Despite the lack of selective $5HT_{1B}$ receptor agonists and antagonists,

stimulation of the $5HT_{1B}$ terminal autoreceptor with non-selective compounds consistently results in a reduction of 5HT release in the frontal cortex and the hippocampus and reduced 5HT synthesis and turnover in the DRN neurons. These results are consistent for $5HT_{1B}$ receptor agonists administered systemically or locally in the cortex or hippocampus [Middlemiss and Hutson, 1990]. Although non-selective antagonists of the $5HT_{1B}$ receptor do inhibit agonist effects, there are no truly selective $5HT_{1B}$ receptor antagonists available [Chopin et al., 1994; Hjorth et al., 1996; Middlemiss and Hutson, 1990; Saito et al., 1996]. Behavioral effects stemming from $5HT_{1B}$ receptor stimulation are unclear since some agonists increase locomotion, while others reduce locomotor activity [Middlemiss and Hutson, 1990].

The neurochemical and behavioral effects of stimulating and blocking some of the other 5HT receptors have also been characterized. Generally, the effects elicited by stimulation of the $5HT_{1D}$ receptor are similar to those of the $5HT_{1B}$ receptor, as expected due to their subunit homology and distribution in the brain [Saito et al., 1996]. Activation of $5HT_2$ receptors with a non-selective agent inhibits DRN 5HT neuron activity and decreases 5HT release in the frontal cortex and striatum; $5HT_2$ receptor antagonists

activate the 5HT system [Saito et al., 1996]. A set of behavioral effects is elicited with $5HT_2$ receptor agonists which is similar to, but less pronounced, than the $5HT_{1A}$ serotonergic syndrome described above [Hillegaart et al., 1996]. $5HT_2$ agonist-induced behavior is blocked by $5HT_2$ antagonists, which include some antipsychotic drugs [Hillegaart et al., 1996]. $5HT_3$ receptors stimulate 5HT release, unlike the $5HT_1$ and $5HT_2$ receptors. This stimulated release has been recorded in the hippocampus, and, since $5HT_3$ antagonists alone do not alter 5HT release, the $5HT_3$ receptors do not appear to mediate a tonic inhibitory 5HT control [Saito et al., 1996]. There is some controversy about the postulated postsynaptic location of this receptor and some researchers suggest that the $5HT_3$ receptor may also function as an autoreceptor [Saito et al., 1996]. A $5HT_4$ receptor agonist, renzapride, increased the 5HT release *in vivo* from the hippocampus after either systemic or local injections [Ge and Barnes, 1996]. This 5HT release was inhibited by the selective $5HT_4$ antagonist (GR113808) which, when administered alone, further reduced basal 5HT release [Ge and Barnes, 1996]. The $5HT_6$ receptor has a high affinity for many antipsychotic drugs, including clozapine, and it has been suggested to play a role in regulating cholinergic transmission [Bourson et al., 1995; Roth et al., 1994]. Recent studies on the $5HT_7$ receptor

subtype have found an abundance of $5HT_7$ receptor mRNA in the hypothalamus, hippocampus and limbic regions. This receptor subtype shows significant affinity for a number of psychotherapeutic drugs [Boess and Martin, 1994; Shen et al., 1993; Sleight et al., 1995]. In addition, *in vitro* studies have shown that the $5HT_7$ receptor binds the putative $5HT_{1A}$ receptor agonist, 8-OHDPAT [Kawahara et al., 1994; Tsou et al., 1994]. There is a paucity of data concerning the function of the $5HT_5$ receptors.

C.3. 5HT and Schizophrenia

Disruptions of the serotonergic system which have been linked to the etiology of schizophrenia are limited to date [Breier, 1995; Chojnacka-Wojcik, 1995; Ohuoha et al., 1993; Rao and Moller, 1994]. The efficacy of antipsychotics with 5HT receptor antagonist activity suggests there may be a 5HT deficiency or abnormal receptor populations [Meltzer, 1995]. Most commonly, a decrease in $5HT_{2A}$ and/or $5HT_{2C}$ receptors and $5HT_{2A}$ receptor binding, and an increase in $5HT_{1A}$ receptor binding is among the findings from clinical studies and autoradiographic postmortem analysis of schizophrenic patients [Burnet et al., 1996; Dean and Hayes, 1996; Ohuoha et al., 1993]. Uncovering the role of 5HT in schizophrenia might also stem

from studies with antipsychotics, some of which are known to preferentially block 5HT receptors. It is proposed that chronic administration of these drugs leads to a desensitization, and a subsequent upregulation, of the blocked receptors. This neurochemical adjustment would compensate for a 5HT deficiency and might explain the delayed clinical effect seen with many of the antipsychotics [Ohuoha et al., 1993].

D. 5-HT-DA INTERACTIONS

D.1. General Interactions

Considering the overlapping neural circuits of serotonergic and dopaminergic systems (Figures 2 and 5), there is obviously a potential for interaction between them. Since the importance of these interactions in the pathophysiology of the psychiatric disorders was established, there have been numerous attempts to characterize the association between the two systems. To date, there is evidence of 5HT-DA interactions in terminal regions, including the striatum, the NAcc, and the hippocampus, as well as in the midbrain at the VTA, SN, and DRN. In fact, it is likely that the two neurotransmitters co-regulate their release throughout the brain. These two systems could interact directly (Figure 6) or indirectly, *via* non-5HT and non-DA neurons. Either way, these connections are likely receptor-based and occur at axo-axonic or axo-somatodendritic sites [Kalivas, 1993].

Electrophysiological studies have linked the DRN to midbrain DA activity. Recordings have been made of decreased activity of single mesoaccumbal DA neurons following direct DRN stimulation [Kelland et al., 1993], and

stimulation of the DRN inhibits the slow firing DA neurons in the nigrostriatal system [Kelland et al., 1990]. Furthermore, in microdialysis studies, intra-NAcc 5HT produced a corresponding dose-dependent increase in extracellular NAcc DA concentrations [Parsons and Justice, 1993], and endogenous 5HT is postulated to positively regulate striatal DA release [Yadid et al., 1994].

D.2. 5HT Regulation of DA

5HT-containing neuronal projections to the striatum, NAcc, VTA and SN are involved in regulating the DA systems at these sites. Given alone, 5HT facilitates DA release from SN and VTA slices. A unique finding from a recent *in vitro* study is that 5HT and $5HT_2$ receptor agonists potentiate the DA feedback inhibition on the DA neurons in the VTA [Brodie and Bunney, 1996]. *In vivo*, stimulation of $5HT_{1A}$, $5HT_{1B}$, $5HT_3$ or $5HT_4$ receptors or blockade of $5HT_{2A/2C}$ receptors increases VTA cell activity and DA turnover [Andersson et al., 1995; Bencoulif et al., 1993; Jiang et al., 1990; Kalivas, 1993; Palfreyman et al., 1993; Saito et al., 1996]. Intra-VTA infusion of 5HT increases DA release in the NAcc, an effect which is not mediated by $5HT_{1A}$ receptors of the VTA [Guan and McBride, 1989]. The interaction is

more complex in the striatum. *In vitro*, 5HT decreases DA release at striatal slices. In contrast, microdialysis studies following intra-striatal and intra-cortical administration of $5HT_{1A}$, $5HT_{1B}$, $5HT_3$ and $5HT_4$ receptor agonists show an increase in DA release in these areas [Consolo et al., 1996; Nomikos et al., 1996; Saito et al., 1996]. A more recent study attributed the striatal DA release to only the $5HT_4$ receptor subtype [Bonhomme et al., 1995]. Of the 5HT receptor subtypes, only the $5HT_{2A/2C}$ receptor appears to mediate a tonic inhibitory control of DA release in the striatum [Saito et al., 1996]. This finding is supported by analyzing compounds with predominantly $5HT_{2A/2C}$ receptor antagonist actions, such as ritanserin and amperozide, which increase DA release in the NAcc [Marcus et al., 1996]. Studies of $5HT_{1A}$ receptor agonists show that they enhance hippocampal and striatal DA turnover [Arborelius et al., 1993; Gobert et al., 1995]. It has been suggested that this may be through a DA_2 receptor blockade, although this is controversial [Gobert et al., 1995; Hjorth et al., 1983]. Based on the studies described above, 5HT may regulate DA activity in the mesocorticolimbic and nigrostriatal systems.

D.3. DA Regulation of 5HT

Comparably, DA regulates the serotonergic system in the brain. *In vitro* studies demonstrate that a non-selective DA receptor agonist stimulates 5HT release in rat SN and hippocampus. This effect may not be DA receptor-mediated, however, since the DA agonist also blocked 5HT reuptake in the SN, which may explain the 5HT increase noted [Saito et al., 1996]. Subsequent studies have indicated that a presynaptic DA_1 receptor in the hippocampus and SN is responsible for the enhanced 5HT release [Saito et al., 1996], while others postulate that it is a D_2 DA receptor-mediated event [Matsumoto et al., 1996]. Another possibility is that DA regulates 5HT release indirectly through DA receptors on adjacent non-5HT neurons [Ferre and Artigas, 1993]. At the origin of the 5HT system, DRN cells seem to be under a DA concentration-dependent inhibition mediated by D_2 DA receptors: the systemic or intra-DRN injection of a D_2 DA receptor agonist increases somatodendritic 5HT release, and decreases striatal 5HT release [Ferre and Artigas, 1993]. As described above for the regulation of DA by 5HT, these results suggest that DA may reciprocally play a role in governing 5HT activity in the midbrain raphé nucleus, and in the mesocorticolimbic and nigrostriatal DA terminals.

There is considerable evidence, then, which implies that an intimate connection exists between the central dopamine and serotonin systems. Inter-regulation of these neurotransmitters supports the notion that the chemical environment in the brain is a very tightly controlled. Characterizing the associations of the DA and 5-HT systems would contribute to a better understanding of the brain circuitry in general. It is also important to functionally define this interaction in order to further elucidate the neurochemical imbalances underlying the psychiatric disorders and thereby, improve treatment possibilities.

E. NICOTINE

E.1. General Nicotine Interactions with the Mesolimbic Pathway

The VTA-NAcc pathway has been identified as one of the neural systems involved in mediating the rewarding effects of stimulant drugs such as cocaine, amphetamine and nicotine. Repeated intermittent or daily administration of these agents results in hyperdopaminergic tone in this pathway, and an increase in locomotor behavior. Tolerance does not appear to develop to this sensitization [Clarke and Kumar, 1983a; Nisell et al., 1996; Parsons and Justice, 1993], however tolerance does develop with chronic infusion [Benwell and Balfour, 1995]. According to these studies, the time course of administration may determine whether tolerance will develop to the locomotor activating effects of these stimulant drugs.

E.2. Nicotine Hyperactivity Model

Based on these distinctive results, a well-characterized animal behavioral model used in research is the nicotine hyperactivity model described by Clarke and Kumar in 1983 [Clarke and Kumar, 1983b]. Using this model in rats, repeated injections of intermittent nicotine leads to a sensitization of the

response of DA neurons (projecting from the VTA to the NAcc) to nicotine. Repeated intermittent nicotine administration increases DA release in the NAcc which produces a concurrent increase in the locomotor activity of the rat. The hyperactivity is a nicotinic receptor-mediated event, mainly in the VTA, since it is effectively blocked by the nicotinic antagonist, mecamylamine [Clarke and Kumar, 1983b; Reavill and Stolerman, 1990].

This may be a useful model with which to study interactions of DA and 5-HT since 5-HT neurons from the DRN are known to directly synapse in the VTA [Hervé et al., 1987]. Furthermore, it is a well-defined and site-specific neurological paradigm in which there is a well-defined behavioral response. Investigation of 5HT receptor-related compounds in this model may be useful for assessing the role of specific receptors in regulating dopaminergic tone in the mesolimbic pathway.

Nicotine has widespread effects in the brain, both behaviorally and neurochemically. To qualify the DA-5HT interaction results of this study, therefore, it is valuable to consider the regional pharmacology and the receptor for nicotine. The pharmacological studies using this nicotine

hyperactivity model will be reviewed in the following sections.

E.3. Nicotinic Receptors

There are nicotinic acetylcholine receptors (NAChR) throughout the brain, and particularly in the NAcc, the VTA, striatum, thalamus and hippocampus [Clarke et al., 1985; Schwartz et al., 1984]. Nicotinic ACh receptors have been identified on DA cell bodies and on DA, 5HT and NE terminals [Lapin et al., 1989]. The widespread distribution of nicotinic ACh receptors implies that there may be diffuse effects of nicotine in the brain. Nevertheless, there is a substantial body of evidence to suggest that nicotine preferentially activates the mesolimbic DA system [Clarke et al., 1988].

The nicotinic receptor is a pentameric ion channel which is composed of α and β subunits. There are multiple subtypes of the NAChR based on its variable composition of subunits. In the mesolimbic system, however, the predominant receptor identified is the $\alpha_4 \beta_2$. There is also a possible role of the $\alpha_3 \beta_2$ subtype in the activation of the mesolimbic system since the nicotinic receptor antagonist α-bungarotoxin, which expresses some selectivity for this receptor subtype, blocks *in vitro* DA release in the VTA-

NAcc pathway [Nisell et al., 1996]. Nicotinic ACh receptors can exist in different functional states: resting, open while conducting ions and desensitized. The desensitized state seems to have the highest affinity for the agonist, and long-term exposure to nicotine induces a desensitized receptor population. Consequently, there are fewer functional nicotinic ACh receptors available, which induces an increase in the number of receptors expressed [Marks et al., 1992]. One theory is that, over the long term, repeated intermittent injections of low dose of agonist will desensitize enough receptors to result in an increase in the total number of receptors, but the low dose will also permit many receptors to regain their level of excitability and thereby activate the nicotinic system [Dani and Heinemann, 1996; Wonnacott, 1990]. The number of α_4 and β_2 subunits is not altered in this process, just the total number of nicotinic ACh receptors [Pauly et al., 1996].

E.4. Behavioral Effects of Nicotine

As described above, repeated intermittent injection of nicotine sensitizes the VTA-NAcc pathway, and leads to a corresponding increase in DA release in the NAcc and in locomotor activity [Clarke et al., 1988]. This behavioral

sensitization has been consistently reported (using 0.1-0.8 mg/kg sc, 5-21 d) [Benwell and Balfour, 1992; Clarke et al., 1988; Clarke and Kumar, 1983a; Clarke and Kumar, 1983b; Ksir, 1994; Reavill and Stolerman, 1990].

Similar increased locomotor effects are seen following acute intra-VTA infusions of nicotine (8 μg bilaterally) or of the nicotinic receptor agonist, cytisine (3.2μg bilaterally) in repeated intermittent nicotine-treated rats, and with repeated intermittent injections of intra-VTA nicotine administration (2.0 μg bilaterally every 2 days) [Panagis et al., 1996]. Acute intra-VTA infusions of cytisine (0.1-10 nmoles/side) also significantly increase locomotor activity, an effect which is blocked by the nicotinic receptor antagonist, mecamylamine (2 mg/kg ip, 20 min latency) [Museo and Wise, 1989].

The behavioral effects of nicotine administered into other brain regions of nicotine-sensitized rats are controversial. There are reports of hyperactivity due to bilateral intra-NAcc nicotine (100,200 μg) or cytisine (30,60 μg) [Benwell and Balfour, 1995; Fung, 1990], and there are other groups who did not detect any behavioral alterations following nicotine injections into the

NAcc, striatum or dorsal hippocampus of pre-sensitized rats [Reavill and Stolerman, 1990]. Repeated intra-NAcc nicotine infusions also lead to a delayed desensitization of the behavioral responses of the mesocorticolimbic DA system [Benwell and Balfour, 1995].

Regardless of the route of administration, all nicotine-induced hyperactivity can be dose-dependently blocked by the nicotinic antagonist, mecamylamine (0.1-2.0 mg/kg sc) [Clarke and Kumar, 1983a; Clarke and Kumar, 1983b; Fung, 1990; Reavill and Stolerman, 1990]. Nicotinic behavioral effects are also blocked by intra-NAcc infusion of the neurotoxin, 6-OHDA (12 μg bilaterally), which damages DA afferents to the NAcc [Clarke et al., 1988; Ksir and Cline, 1987] . Another group, however, found that neonatally administered 6-OHDA does not prevent locomotor activating effects of nicotine, suggesting that DA may not be imperative for locomotor activity [Vezina et al., 1994]. Based on these studies, DA is important in the regulation of locomotor activity in the intact animal and it has been suggested that the conflicting neonatal results might be the result of an adaptive change to the developmental insult [Rogers and Dunnett, 1989]. These results suggest that nicotine is likely acting primarily at nicotinic ACh receptors in

the VTA to induce sensitization in DA projections to the NAcc.

In addition to hyperactivity, nicotine affects other behavioral parameters, including rearing and stereotypy. A recent analysis of behavioral effects of acutely and repeated intermittently administered nicotine showed that nicotine (0.1-0.4 mg/kg, 5d) dose-dependently decreases rearing activity, and it dose-independently increases stereotypy, compared to saline control rats [Ksir, 1994]. In contrast, a recent study of repeated intermittent nicotine administration (0.5 mg/kg sc, 12 d) reported increases in all measured locomotor parameters except rearing [Nisell et al., 1996].

E.5. Neurochemical Effects of Nicotine

Neurochemically, there is evidence that repeated intermittent nicotine administration results in an increased DA release. Nevertheless, in considering the present effects of 5HT receptor ligands on DA regulation using the nicotine hyperactivity model, it is important to recognize the potential interactions of nicotine with the DA and 5HT neurotransmitter systems. The observed effects of nicotine may be due to its direct and indirect actions on the DA or 5HT systems. One study found that repeated

intermittent injections of nicotine (0.4 mg/kg, 40 d) selectively reduced 5HT and 5HIAA levels in the hippocampus [Benwell and Balfour, 1992], whereas acute nicotine (0.4 mg/kg sc) did not change NAcc 5HIAA levels [Mirza et al., 1996]. In addition, acute nicotine (0.6 mg/kg sc) preferentially releases DA from the NAcc compared to the CNuc [Di Chiara and Imperato, 1988; Imperato et al., 1986; Joseph et al., 1993]. Furthermore, many studies have detected the expected increase in NAcc DA following repeated intermittent injections of nicotine injections. Using microdialysis techniques to study the effects of nicotine on the VTA-NAcc pathway, researchers recorded an increase in NAcc DA release and VTA neuronal activity in animals following repeated intermittent injections (0.5 mg/kg sc, 12 d) and acute treatment paradigms [Nisell et al., 1996]. The NAcc DA release due to repeated intermittent injections of nicotine (0.8 mg/kg sc 10 x/14 d) is lower than that measured in acutely-treated animals [Lapin et al., 1989]. In contrast, another group detected a more marked increase in NAcc extracellular DA release following repeated intermittent injections (0.4 mg/kg, 5 d) than acute nicotine (0.4 mg/kg sc) administration [Benwell and Balfour, 1992]. In this same study, however, there was no significant change detected in the NAcc DA levels with tissue analysis after a repeated intermittent nicotine treatment,

which suggests that post mortem tissue levels, as used in the current study, may not give an accurate indication of nicotine-induced DA changes.

As with the behavioral effects, however, the neurochemical changes induced by nicotine might also be a function of the sampling time and the type of administration since repeated intermittent injections of nicotine produced higher NAcc extracellular DA levels than chronic infusion techniques [Benwell et al., 1995]. In this case, the infused animals did eventually express elevated NAcc DA levels once the nicotine treatment ended. Compared to acute regimens, the neurochemical effects of repeated intermittent injections of nicotine treatment are unclear. There is increased nicotinic ACh receptor radioligand binding in the rat brain following repeated intermittent injections of nicotine administration, specifically in the striatum, dorsal hippocampus, frontal cortex, the entorhinal cortex and the cerebellum [Abdulla et al., 1996; Robinson et al., 1996; Yates et al., 1995]. Finally, the method of analysis used will significantly affect the neurochemical results obtained. For example, *ex vivo* analysis, as used in the current experiments, measures the total (intracellular and extracellular) level of neurochemicals in the tissue being studied whereas *in vivo* microdialysis

techniques provide a sampling of the extracellular neurochemical concentrations in selected areas within a brain region. As such, results obtained using *ex vivo* analytical techniques are limited compared to those from *in vivo* microdialysis studies.

The effect of nicotine on 5HT and DA metabolism has also been studied. Acute nicotine (0.8 mg/kg sc) does induce DA turnover and clearance in the NAcc compared to cortical, VTA, SN and CNuc areas, but tolerance develops to these metabolic effects with repeated administration of the drug (0.4 and 0.8 mg/kg daily 5d/wk, 6 wk) [Ksir et al., 1995; Lapin et al., 1989]. Accordingly, acute nicotine administration (0.4 mg/kg sc) leads to increases in NAcc DOPAC and HVA levels, and to increased DOPAC and DA levels [Benwell and Balfour, 1992; Mirza et al., 1996]. In contrast, repeated intermittent administration of nicotine (0.4 mg/kg, 5d) appears to decrease NAcc DA turnover based on decreased basal DOPAC levels and increased DA levels. Tolerance does not appear to develop to the increased metabolic effects of *l*-nicotine with repeated exposure (0.2-0.4 mg/kg sc, 12 d) in some mesolimbic regions, including the olfactory tubercle and NAcc [Clarke et al., 1988]. In addition, high doses of acute nicotine (2.0 mg/kg x 3-4/2 h)

increase the whole brain 5HT turnover rate. Tolerance also develops to this neurochemical effect with repeated intermittent injections of nicotine.

According to the nicotine hyperactivity model, nicotine may sensitize the mesolimbic pathway by binding to nicotinic ACh receptors at the VTA. In support of this proposal, nicotinic ACh receptors in the VTA seem to be more important in regulating the mesolimbic DA tone than those in the NAcc since the only intra-VTA, not intra-NAcc, administration of the nicotinic receptor antagonist, mecamylamine, blocks the nicotine-induced increase in DA release in the NAcc [Nisell et al., 1995].

F. 5HT-RECEPTOR RELATED LIGANDS

The experiments described in this thesis involved systemic injections of 5HT receptor-related compounds to view the general effects of these drugs on the mesolimbic DA activity. This analysis was limited to the $5HT_{1A}$, $5HT_{1B/D}$, and $5HT_{2A/C}$ receptor subtypes. There are several selective and non-selective ligands which have been characterized for these specific 5HT receptors, but only those listed in table 3 are used in the following study. The dose of each compound was chosen based on the behavioral effects elicited by a similar dose reported in the literature.

Table 3: 5HT Receptor Ligands Used in This Study

Receptor	Agonists	Antagonists
$5HT_{1A}$	8-OHDPAT	WAY 100635
	NAN 190 (partial)	
$5HT_{1B}$	TFMPP	Pindolol
	RU 24969	
$5HT_2$		Ritanserin

Throughout this thesis, stereospecific drug preparations are racemic unless otherwise mentioned.

F.1. $5HT_{1A}$ Receptor Ligands

Three compounds were used to study the role of the $5HT_{1A}$ receptor in regulating DA neural activity; including a selective agonist, 8-OHDPAT, a putative partial agonist, NAN 190, and an antagonist, WAY 100635.

F.1.1. 8-OHDPAT

8-OHDPAT [8-hydroxy-2-(di-*n*-propylamino)tetralin] is a well-studied, selective $5HT_{1A}$ receptor agonist [Hillegaart et al., 1996]. Since it has a high affinity for $5HT_{1A}$ (pk_i=9.0 in rats) receptors [Millan et al., 1995], 8-OHDPAT is used as a ligand in radioactivity binding studies of this receptor, with a binding profile mainly at DRN and MRN cell bodies and fibers [Zwicker et al., 1996]. In addition, it has recently been found to have affinity for the $5HT_7$ receptor subtype [Kawahara et al., 1994; Tsou et al., 1994]. 8-OHDPAT also has an affinity for D_2 DA receptors, more so at higher doses [Millan et al., 1995]. This compound is optically active and studies done on the R and S enantiomers have found that generally, (R)-8-OHDPAT has a more potent $5HT_{1A}$ receptor agonist effect [Hadrava et al., 1996].

8-OHDPAT binds to somatodendritic 5HT autoreceptors at the DRN and

binds to postsynaptic 5HT receptors at the hippocampus and striatum [Nomikos et al., 1992]. At higher doses, 8-OHDPAT stimulation of postsynaptic $5HT_{1A}$ receptors may be responsible for the observed "serotonergic syndrome"; the $5HT_{1A}$ receptor antagonists (S)-UH-301 and the novel compounds WAY 100635 (1mg/kg) and WAY 100135 (10 mg/kg) reverse and reduce this 5HT syndrome, respectively [Fletcher et al., 1995; Mundey et al., 1996; Nomikos et al., 1992]. Administration of 8-OHDPAT at doses ranging up to 0.4 mg/kg (sc) induced this behavioral effect, which includes stereotyped forward movement and a decrease in total activity and rearing counts [Hillegaart et al., 1996]. Some studies have observed hyperactivity following administration of 8-OHDPAT [Kalkman and Soar, 1990; Tricklebank et al., 1984], and others report decreased spontaneous motor activity following acute 8-OHDPAT [Hillegaart et al., 1989; Mittman and Geyer, 1989]. In mice, however, 8-OHDPAT (0.5-5.0 mg/kg) induced a decrease in locomotor activity. This hypoactivity was only blocked by DA ligands, including the D_2 DA receptor antagonist, spiperone, and the non-specific DA receptor antagonist haloperidol, but not by the 5HT ligands NAN 190 (0.5-2 mg/kg), pindolol or metergoline (a non-selective 5HT receptor antagonist) [Chojnacka-Wojcik, 1992]. This pharmacological

behavioral profile suggests that, in mice, the locomotor effect of 8-OHDPAT may not be due to its action on 5HT receptors, but to DA receptor stimulation [Chojnacka-Wojcik, 1992].

8-OHDPAT administration produces a range of neurochemical alterations in the central serotonergic system. Administered systemically, 8-OHDPAT inhibits neuronal firing and 5HT release from the DRN, and reduces 5HT turnover in the striatum [Gobert et al., 1995; Millan et al., 1995; Nomikos et al., 1992]. Conversely, 8-OHDPAT enhanced 5HT turnover in the hippocampus, which could be blocked by (S)-UH-301 [Matos et al., 1996]. 8-OHDPAT (0.1 mg/kg sc) also attenuates 5HT synthesis in the striatum and limbic forebrain, an effect which is reversed by cerebral hemisection, suggesting that this inhibition is mediated by the drug's agonist actions at presynaptic $5HT_{1A}$ autoreceptors in the DRN [Hjorth et al., 1996]. In contrast, since the 8-OHDPAT-induced increase in cortical ACh release is blocked only by a $5HT_{1A}$ receptor antagonist (WAY 100635) and a DA_1 receptor antagonist (SCH 23390), it has been suggested that this drug also activates postsynaptic $5HT_{1A}$ receptors. The hypothesis is that stimulating $5HT_{1A}$ receptors leads to a concurrent increase in DA release which, *via* DA_1

receptors, stimulates the observed ACh release [Consolo et al., 1996]. One well-characterized effect is the decreased extracellular 5HT levels in the hippocampus and striatum following systemic 8-OHDPAT administration [Assie and Koek, 1996a; Fletcher et al., 1995; Ge and Barnes, 1996; Matos et al., 1996; Nomikos et al., 1992; Saito et al., 1996]. These inhibitory effects are reversed by administration of the $5HT_{1A}$ receptor antagonists (S)-UH-301 and WAY 100635 (1mg/kg) [Arborelius et al., 1994; Fletcher et al., 1995; Mundey et al., 1996; Nomikos et al., 1992], and by the non-selective $5HT_{1A}$ receptor antagonists (±)-propranolol and (±)-pindolol [Matos et al., 1996].

As with systemic administration, intra-DRN injections of 8-OHDPAT also decrease striatal and hippocampal 5HT levels in dialysate, and decrease 5HT turnover in the NAcc and striatum [Hutson et al., 1989; Invernizzi et al., 1991; Saito et al., 1996]. In contrast to systemic and DRN administration, injections of 8-OHDPAT into the hippocampus and striatum do not affect the local 5HT levels [Nomikos et al., 1992; Saito et al., 1996], or significantly increase the extracellular 5HT levels in the hippocampus, an effect which is not blocked by WAY 100635 [Assie and Koek, 1996b]. One proposal for the observed increase in hippocampal 5HT levels is that it may be due to the

ability of 8-OHDPAT to inhibit 5HT reuptake sites. Finally, intra-hippocampal infusions of 8-OHDPAT increased local ACh release [Cassel and Jeltsch, 1995]. Hippocampal $5HT_{1A}$ receptor stimulation apparently did not affect the mesocorticolimbic DA system since alterations in NAcc DA release were not recorded [Jiang et al., 1990].

The neurochemical effect of 8-OHDPAT on midbrain DA systems has also been investigated. Some studies have found that 8-OHDPAT does not alter NAcc or dorsal striatal DA levels [Arborelius et al., 1993; Jiang et al., 1990], while other studies have found that it does increase levels of the DA metabolites DOPAC and HVA in the hippocampus [Matos et al., 1996]. These effects may be dose-dependent since, at lower doses, systemic administration results in a greater increase in DA activity in the VTA compared to the nigrostriatal system, but at high doses 8-OHDPAT produces a decrease in DA released in both of these areas [Arborelius et al., 1993; Cornfield et al., 1991]. Intra-NAcc and intra-striatal infusion of low doses (1-10 μM) had no effect on the local DA levels [Nomikos et al., 1996] compared to the increase in DA release following systemic administration of 8-OHDPAT [Benclouif et al., 1993]. This $5HT_{1A}$ receptor agonist also alters

the regional neurotransmitter metabolic rate. 8-OHDPAT (0.6 mg/kg sc) significantly, and dose-dependently decreases DA turnover in the hippocampus as measured by a decrease in DOPAC levels by HLPC-ED [Gobert et al., 1995], although in other reports it increases DA turnover, preferentially in the ventral striatum [Arborelius et al., 1993]. Alternatively, the elevated DA levels may be due to 8-OHDPAT interactions with the $5HT_{1A}$ heteroreceptor, which has recently been identified on DA terminals in the striatum. *In vitro*, 8-OHDPAT stimulation of the rat $5HT_{1A}$ receptor results in inhibition of tyrosine hydroxylase, the rate limiting enzyme in DA synthesis [Johnson et al., 1996]. This would be expected to yield a decrease in DA levels.

F.1.2. NAN 190

The effects of a second $5HT_{1A}$ receptor agonist, NAN 190, were also studied in these experiments. NAN 190 (1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl] piperazine) has been characterized as a selective $5HT_{1A}$ receptor partial agonist (Ki = 0.58 nM) since it has only modest affinity for other 5HT receptor subtypes [Middlemiss and Tricklebank, 1992]. It also

has a modest affinity for α_1 receptors [Glennon et al., 1988; Middlemiss and Tricklebank, 1992]. In general, its neurochemical actions on the serotonergic system are as a partial agonist at presynaptic $5HT_{1A}$ autoreceptors, and as an antagonist at postsynaptic $5HT_{1A}$ receptors. Accordingly, at presynaptic sites, systemic and intra-DRN injections of NAN 190 decrease the 5HT release at the DRN and into the ventral hippocampus, whereas, postsynaptically, it antagonizes the 8-OHDPAT-induced serotonergic syndrome [Nomikos et al., 1992]. These opposite effects appear to be dose-dependent since, even at very low doses, partial agonism at somatodendritic autoreceptors is seen, but postsynaptic antagonism only occurs at higher doses. *In vivo* studies support this suggestion since low doses of NAN 190 (up to 1 mg/kg), like the $5HT_{1A}$ receptor agonist 8-OHDPAT, decreased 5HT synthesis in the frontal cortex, 5-HIAA levels in the DRN, and 5HT release in the hippocampus [Claustre et al., 1991; Nomikos et al., 1992; Sharp et al., 1996]. Consistent with the proposed $5HT_{1A}$ receptor agonist action of NAN 190, a NAN 190-induced (0.03-0.3 mg/kg) decrease in hippocampal 5HT release was blocked by the selective $5HT_{1A}$ antagonists WAY 100135 and WAY 100635 [Gartside et al., 1995; Sharp et al., 1996]. These workers suggest that agonist effects are not seen at higher doses due to the drug's non-

specific effects.

Systemically, NAN 190 (3.9 μg/kg iv) attenuated DRN neuronal firing, which suggests its action as a somatodendritic autoreceptor antagonist [Gobert et al., 1995]. NAN 190 has also been found to dose-dependently inhibit 5HT and DA turnover in the rat striatum and hippocampus [Gobert et al., 1995]. Unlike the effects of 8-OHDPAT, *in vitro* stimulation of $5HT_{1A}$ receptors with NAN 190 (10 μM) does not inhibit striatal tyrosine hydroxylase activity [Johnson et al., 1996]. A radioligand binding study showed that NAN 190 and 8-OHDPAT have the same binding profile at postsynaptic sites in the hippocampus, striatum, and frontal cortex, and that NAN 190 can competitively antagonize adenylyl cyclase inhibition induced by $5HT_{1A}$ receptor agonists in the hippocampus [Rydelek-Fitzgerald et al., 1990]. In addition, NAN 190 competitively antagonized the *in vitro* inhibition of adenylyl cyclase in hippocampal membranes by the non-selective $5HT_{1A}$ receptor agonist, 5-carboxamidotryptamine (5-CT). In support of its purported partial agonist role, NAN 190 demonstrated a weaker affinity for $5HT_{1A}$ receptor GTP activation than did 8-OHDPAT [Assie and Koek, 1996a].

Behaviorally, the effects of NAN 190 are complex. The results of some studies show that, at lower doses (up to 1 mg/kg), NAN 190 induces the serotonergic syndrome [Schreiber et al., 1995; Ybema et al., 1993], while other studies detect only antagonistic effects on the same behaviors [Glennon et al., 1988; Nomikos et al., 1992]. One theory is that as a partial $5HT_{1A}$ receptor agonist, higher doses of NAN 190 might be expected to antagonize the serotonergic syndrome.

F.1.3. WAY 100635

The effects of antagonizing the $5HT_{1A}$ receptor were investigated using a novel, selective $5HT_{1A}$ receptor antagonist, WAY 100635 (N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl)cyclohexane carboxamide trihydrochloride) [Fletcher et al., 1995]. This compound is a competitive and selective $5HT_{1A}$ receptor antagonist, with a 100-fold affinity for this receptor over any others [Forster et al., 1995]. As a radioligand, it has the same binding profile as 8-OHDPAT, which suggests that it binds at somatodendritic autoreceptors, and at postsynaptic $5HT_{1A}$ receptors. This hypothesis is supported by many studies [Bosker et al., 1996; Fletcher et al.,

1995; Forster et al., 1995; Mundey et al., 1996]. Accordingly, *in vitro* and *in vivo* studies show that WAY 100635 dose-dependently blocks the effects of $5HT_{1A}$ receptor agonists at hippocampal postsynaptic sites and at somatodendritic autoreceptors in the DRN [Fletcher et al., 1995; Sharp et al., 1996]. In addition, the postsynaptic $5HT_{1A}$ receptor-stimulated DA release is also blocked by WAY 100635 [Consolo et al., 1996; Fletcher et al., 1995]. In a study using guinea pigs, WAY 100635, given alone, increased the activity of DRN neurons, which supports the theory that 5HT exerts a tonic inhibitory control on DRN activity *via* $5HT_{1A}$ autoreceptors [Mundey et al., 1996]. Behaviorally, WAY 100635 alone does not elicit any effects, but it prevents the $5HT_{1A}$ receptor agonist-induced serotonergic syndrome even at very low doses (0.003 mg/kg) [Fletcher et al., 1995; Forster et al., 1995; Mundey et al., 1996; Sharp et al., 1996].

F.2. $5HT_{1B}$ Receptor Ligands

In comparison to the extensive pharmacology of the $5HT_{1A}$ receptor, there is a limited range of selective ligands for the $5HT_{1B}$ receptor available. To study the role of this receptor in mesolimbic DA regulation, three non-

selective agents were employed in this study: two $5HT_{1B}$ receptor agonists, TFMPP and RU 24969, and the $5HT_{1B}$ receptor antagonist, (±)-pindolol. At the time of experimentation, TFMPP was judged to be a reasonable choice for a $5HT_{1B}$ agonist, however subsequent studies were carried out with the more selective $5HT_{1B}$ receptor-related compound, RU 24969.

F.2.1. TFMPP

TFMPP (α,α.α-trifluoromethylphenylpiperazine) is a piperazine compound with a high affinity for the $5HT_{1B}$ receptor. It is a non-specific serotonergic compound since it also has a high affinity for the $5HT_{1A}$, $5HT_{2C}$, and the $5HT_{1D}$ receptors [Middlemiss and Hutson, 1990]. Since the $5HT_{1B}$ receptor appears to only be found in mice and rats, TFMPP studies at this receptor have been limited to these two animal models. TFMPP decreases locomotor activity of rats (2-20 mg/kg systemically), and these effects are only blocked by a non-selective $5HT_1$ or $5HT_2$ antagonist [Lucki et al., 1989; Middlemiss and Hutson, 1990]. Furthermore, the suppression of locomotor activity by a similar piperazine agonist, m-CPP, is potentiated by the administration of a 5HT-neurotoxin and attenuated by elevated 5HT levels. These results suggest that m-CPP and TFMPP likely induce feedback inhibition by acting

at presynaptic 5HT autoreceptors, namely $5HT_{1B}$ receptors, or regulate locomotor activity by interacting with postsynaptic $5HT_{2C}$ receptors [Lucki et al., 1989]. In the rat, microdialysis studies show a decreased 5HT release from the frontal cortex and hippocampus after local or systemic injections of TFMPP in the rat [Saito et al., 1996]. *In vitro*, however, TFMPP increases 5HT release from hippocampal, hypothalamic and diencephalic slices. These drug-induced 5HT increases were blocked by serotonin reuptake blockers, such as imipramine and fluoxetine, suggesting that TFMPP may interact with the 5HT uptake system. This is supported by *in vitro* studies in which TFMPP prevented synaptosomal [^{3}H]5HT uptake [Saito et al., 1996]. Furthermore, TFMPP decreases 5HT synthesis, which is blocked only by the $5HT_{1B}$ receptor antagonists cyanopindolol and methiothepin *in vitro* [Hjorth et al., 1996].

F.2.2. RU 24969

The second $5HT_{1B}$ agonist, RU 24969 [5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole], is available as a succinate salt. It is a novel, relatively-selective $5HT_{1B}$ receptor agonist (pk_i=8.2;8.4), with lower affinities for $5HT_{1A}$ (pk_i=8.1) and $5HT_{1D}$(pk_i=7.3) sites and a weak affinity for other

5HT subtypes, and for DA and noradrenergic receptors [Kalkman, 1995; Tricklebank et al., 1986]. As with TFMPP, systemic or local administration of RU 24969 decreases hippocampal and cortical 5HT release [Middlemiss and Hutson, 1990; Saito et al., 1996; Sharp et al., 1989]. At the DRN, its inhibition of 5HT release is blocked by the $5HT_{1B}$ receptor partial agonists, cyanopindolol and methiothepin, which suggests that RU 24969 is acting as an agonist at terminal $5HT_{1B}$ autoreceptors [Middlemiss and Hutson, 1990]. Alternatively, the drug's effects may be at postsynaptic sites since in a study using 5HT-depleted animals, RU 24969 still induced an increase in locomotor activity [Oberlander et al., 1987]. In addition, RU 24969 decreases 5HT synthesis and turnover in the DRN and striatum, and latently increases 5HT turnover in the NAcc [Green et al., 1984; Middlemiss and Hutson, 1990; Oberlander et al., 1987]. Elevated DA release in the striatum is another neurochemical effect of RU 24969 [Saito et al., 1996], which is blocked by pindolol [Bencoulif et al., 1993].

In behavioral tests, RU 24969 is distinctive for its hyperactivity effects (0.5-36 mg/kg), with an associated increase in repetitive activity and a decrease in rearing activity [Goodwin and Green, 1985; Rempel et al., 1993; Sipes and

Geyer, 1996; Tricklebank et al., 1986]. The increased locomotion is not affected by coadministration with (-)-propranolol, a $5HT_1$ receptor antagonist, but it is blocked by pindolol, a $5HT_{1A}$ and $5HT_{1B}$ receptor antagonist [Bencoulif et al., 1993; Goodwin and Green, 1985; Tricklebank et al., 1986]. The locomotor effects of RU 24969 are pharmacologically similar to those of a known presynaptic 5HT releasing agent, MDMA, and it has been suggested that the effects of both of these agents are mediated through a $5HT_{1B}$ receptor mechanism [Rempel et al., 1993]. The locomotor activating effects of RU 24969 are also seen in animals treated with the 5HT selective neurotoxin, 5,7-dihydroxytryptamine, injected into the DRN [Tricklebank et al., 1986]. This suggests that the locomotor effects of RU 24969 are mediated at postsynaptic receptor sites. In contrast, other reports have suggested that the effects of RU 24969 are mediated by non-$5HT_{1B}$ receptors. In one study, RU 24969-induced increases in locomotor activity were blocked only by the selective $5HT_{1A}$ receptor antagonists WAY 100635 and SDZ 216-525, and not by the selective $5HT_{1B}$ receptor antagonist, GR 127935 [Kalkman, 1995]. Another study found that the $5HT_{2A/2C}$ antagonist ritanserin, but not the $5HT_1$ antagonist (-)-propranolol, enhanced RU 24969-induced hyperactivity [Goodwin and Green, 1985].

F.2.3. Pindolol

In this experiment pindolol [1-(1H-indol-4-yloxy)-3-[(1-methylethyl)amino]-2-propanol], one of the original β receptor blocking compounds, is used as a $5HT_{1B}$ antagonist. In serotonergic systems, it has approximately equal affinity for the $5HT_{1A}$ and $5HT_{1B}$ receptors, and a lower affinity for $5HT_{1D}$ and α sites [Chopin et al., 1994; Matos et al., 1996]. At $5HT_{1B}$ receptors, pindolol acts as a partial agonist but at $5HT_{1A}$ receptor sites, it acts as both a partial agonist and an antagonist [Glennon et al., 1988; Meltzer and Maes, 1996]. It is a chiral compound and, reportedly, the (-)-pindolol enantiomer is more selective for the $5HT_{1B}$ receptor than for $5HT_{1A}$ receptor [Brunivels et al., 1993b]. Cyanopindolol, a chemically modified analogue, is also more selective for $5HT_{1B}$ sites [Middlemiss and Hutson, 1990]. In locomotor activity tests, pindolol alone has no effect and, in combination, it prevents 8-OHDPAT and other $5HT_{1A}$ agonists from eliciting the serotonergic syndrome [Hillegaart et al., 1996]. In addition, studies found that pindolol is effective at altering the behavioral effects of $5HT_{1B}$ receptor agonists, for example attenuating the RU 24969-hyperactivity [Tricklebank et al., 1986]

Neurochemically, local and systemic administration of (-)-pindolol alone (2.5

and 10 mg/kg sc; 10 μM) increased 5HT levels in the rat ventral hippocampus, possibly through both $5HT_{1A}$ and $5HT_{1B}$ receptor antagonism [Assie and Koek, 1996a]. In the nucleus accumbens, co-perfusion of pindolol (4μM) completely antagonizes the 5HT-induced (0.1-0.4 μM) increase in extracellular DA levels [Parsons and Justice, 1993]. In the anterior striatum, co-perfusion of pindolol (4 nmol) blocks the DA release induced by the $5HT_{1B}$ receptor agonist, RU24969 (2 nmol) [Bencoulif et al., 1993]. Intra-DRN perfusion of (-)-pindolol alone increased the local and hippocampal 5HT concentrations [Matos et al., 1996].

Pindolol also has significant neurochemical interactions when coadministered with other pharmacological agents. Systemic pindolol reverses the reduced DRN cell firing and decreased 5HT release from the DRN caused by $5HT_{1A}$ agonists, 8-OHDPAT and buspirone, acting at presynaptic autoreceptors [Assie and Koek, 1996a; Matos et al., 1996]. Postsynaptically, the effects of pindolol are unclear since there is evidence that it blocks the $5HT_{1A}$ agonist effects at post-synaptic receptors in the hippocampus, preventing a reduction in hippocampal 5HT release [Assie and Koek, 1996a; Matos et al., 1996], although other results show that pindolol has no antagonistic effects at

postsynaptic $5HT_{1A}$ sites [Romero et al., 1996]. Intra-DRN pindolol perfusion blocks $5HT_{1A}$ autoreceptors and, therefore, dose-dependently increases 5HT release from the DRN; intra-hippocampal pindolol administration also increases 5HT release [Assie and Koek, 1996a; Matos et al., 1996]. In agreement with behavioral studies, pindolol does block $5HT_{1B}$ receptors in neurochemical tests: in the striatum, administration blocks the increase in extracellular DA levels induced by infusion of the $5HT_{1B}$ receptor agonist RU 24969 [Bencoulif et al., 1993].

F.3. $5HT_2$ Receptor Ligands

The final 5HT receptor studied in the present set of experiments was the $5HT_2$ receptor subtype. As with the $5HT_{1B}$ receptor, there has been a limited availability of compounds which are selective for the $5HT_2$ receptor subtypes. A $5HT_{2A/2C}$ antagonist, ritanserin, is used to study the $5HT_2$-mediated effects on the mesolimbic DA pathway. Ritanserin is classified as a non-specific $5HT_{2A/2C}$ receptor antagonist with an equal affinity for the $5HT_{2A}$ and $5HT_{2C}$ receptor subtypes [Leysen et al., 1985].

Until the recent discovery of $5HT_{2A/2C}$ receptor-mediated changes in

midbrain DA activity, the $5HT_2$ receptor was not believed to play a role in regulating the expression of locomotor activity. Specifically, the administration of non-specific $5HT_{2A/2C}$ receptor agonists induces a stereotyped hyperlocomotor activity comparable to the previously described "serotonergic syndrome" [Hillegaart et al., 1996]. In some previous studies, $5HT_{2A/2C}$ receptor antagonists only had behavioral effects following chronic administration, an effect which parallels the delayed clinical efficacy of antipsychotics with $5HT_{2A/2C}$ receptor antagonist properties [Palfreyman et al., 1993]. In addition, the $5HT_2$ receptor antagonists preferentially affect activated (nicotine-treated animals) compared to resting (saline-treated animals) DA systems in behavioral and neurochemical tests [Palfreyman et al., 1993].

F.3.1. Ritanserin

Microdialysis studies have shown mesolimbic alterations following administration of $5HT_2$ receptor ligands, including inhibition of DA activity with intra-VTA $5HT_2$ receptor agonists [Brodie and Bunney, 1996]. In accordance with these findings, the $5HT_2$ receptor antagonist ritanserin induces increased extracellular and whole tissue DA levels in the NAcc

[Devaud et al., 1992; Marcus et al., 1996]. Ritanserin was also found to increase DA neuronal activity of the VTA and the SN, with a more pronounced effect in the VTA [Andersson et al., 1995]. In comparison to local administration, in one study systemic ritanserin (1.5 mg/kg sc) failed to alter the extracellular DA concentrations in the striatum of rats, and it (1.0 mg/kg iv) did not significantly alter the voltammetric DA signal measured in the NAcc or dorsolateral striatum in anaesthetized rats [Andersson et al., 1995]. Behaviorally, however, systemic administration of high doses of ritanserin (10 mg/kg) resulted in a decrease in locomotor activity [Peltier et al., 1994]

G. HYPOTHESES & AIM OF STUDY

The primary hypothesis in this study is that 5HT inhibits mesolimbic DA activity. Accordingly, an increased serotonergic tone would inhibit DA release and suppress locomotor activity. Stimulation of autoreceptors, such as $5HT_{1A}$ and $5HT_{1B}$ receptors, is thus expected to decrease terminal 5HT release and promote DA activity. In contrast, stimulation of postsynaptic receptors, such as the $5HT_2$ receptor, would be expected to suppress DA activity.

The aim of the study is to investigate the behavioral and neurochemical effects of selected 5HT receptor ligands in a well-characterized model of mesolimbic DA function.

MATERIALS AND METHODS

A. CHEMICALS

TABLE 4: Chemicals Used During This Study

Chemicals	Supplier
3,4-Dihydroxyphenylacetic Acid (DOPAC)	Sigma Chemicals Co. (St.Louis, MO, USA)
5-Hydroxyindole-3-acetic Acid (5HIAA)	Sigma
(±)-8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OHDPAT) HBr	Research Biochemicals Inc. (RBI) (Wayland, MA, USA)
Acetic Acid, Glacial	Fisher Scientific (Fairlawn, NJ, USA)
Acetonitrile 10%, HPLC grade	BDH Inc. (Toronto, ON, Canada)
Ascorbic Acid	Sigma
Dopamine HCl (DA)	Sigma
Ethylenediaminetetraacetate disodium salt (EDTA)	Fisher
Homovanillic Acid (HVA)	Sigma
Hydrochloric Acid (HCl)	BDH
Methanol (MeOH)	BDH
1-(2-methoxyphenyl)-4-[4-(2-pthalimido) butyl]piperazine (NAN 190) HBr	RBI
(-)-Nicotine Hydrogen Tartrate	Sigma
Nitrogen Gas	Praxair (Mississauga, ON, Canada)
Perchloric Acid ($HClO_4$), 60%	Fisher
Phosphoric Acid	Fisher
(±)-pindolol	RBI
Potassium Chloride (KCl)	Fisher

Chemicals	Supplier
5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole (RU 24969)	Roussel-Uclaf (Paris, Fr.)
Saline, Isotonic, 9%	Fisher
Serotonin (5HT) Creatinine Sulfate	Sigma
Sodium Metabisulfite	Fisher
Sodium Phosphate dibasic ($Na_2HPO_4 \cdot 2H_2O$)	Fisher
Sodium Octyl Sulfate (SOS)	Aldrich (Milwalkee, WI, USA)
Sodium Chloride (NaCl)	Fisher
4-(3-triflurormethylphenyl)piperazine (TFMPP) HCl	RBI
Tween 80	Fisher
N-(2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl)-N-(2-pyridinyl) cyclohexane carboxamide trihydrochloride (WAY 100635 (A-5))	Wyeth Ayerst

B. ANALYTICAL INSTRUMENTATION AND APPARATUS

B.1. High Performance Liquid Chromatography with Electrochemical Detection (HPLC-ED)

Brain levels of DA and 5-HT and the acid metabolites DOPAC, HVA and 5HIAA were measured by reverse phase HPLC-ED.

The chromatographic analysis of each sample was performed using a WISP 710B automatic injection system (Waters; Milford,MA, USA) at a flow rate of 1 ml/min (Waters model 510 pump). The volume of each injection was adjusted to 15 µl. The compounds of interest were separated on µbondapak C_{18} precolumn (Waters), followed by a Spherisorb 5 ODS 2 column (250 x 4.6 mm; 5 µm particle size; Phenomenex, Torrence, CA, USA). Column effluents were registered by an electrochemical detector (model M460; Waters) with an applied voltage of 0.80 V. These peaks were recorded and integrated using a model 740 Data Module system (Waters).

The HPLC mobile phase consisted of $Na_2HPO_4 \cdot H_2O$ (55 nM), sodium octyl sulfate (73 nM), EDTA (37 nM) and 10% acetonitrile. This solution was filtered (type HA filter; 0.45 µm; Millipore, Mississauga, ON, Canada), degassed and adjusted to a pH of 3 with phosphoric acid.

B.2. Locomotor Activity System

The locomotion of each rat was measured by an activity monitoring system

(Acadia Instruments Ltd., Saskatoon, SK, Canada) which consists of six 17" x 17" x 12" plexiglass test cages, each containing two parallel infrared grids (12 X 12 beams). Measurements are based on the number of infrared interruptions.

Three parameters were digitally recorded in 5 min intervals over a 30 min period:

a. total ambulatory activity: corresponds to total number of beam breaks

b. stereotyped activity: represented by two or more consecutive interruptions of the same infrared beam

c. rearing activity: represented by number of upper beam breaks.

The results were recorded temporally and stored by a microcomputer system

B.3. Tissue Homogenizer

Tissue samples (caudate nucleus, nucleus accumbens and hippocampus) for HPLC analysis were homogenized using a Tri-R homogenizer (Model S63C, Tri-R Instruments, Rockville, NY, USA) with a Teflon ® pestle and plastic microfuge tube. The rotor shaft had a maximum speed of 12,000 rpm with a ten speed setting; in this study a speed setting of 7 was used

routinely.

B.4. Centrifuges

All centrifugations were performed using a MSE Micro-Centaur benchtop centrifuge (Baxter Corp., Edmonton, AB. Canada) which is suitable for small volume, high speed centrifugation.

B.5. pH Meter

The pH values of the various buffer solutions were determined using an Accumet Model 915 meter (Fisher Scientific, Fairlawn, NJ, USA) standardized with a certified buffer solution (pH 7.00 ± 0.01, 23°C, Fisher Scientific).

B.6. Weighing Balances

Tissue samples and chemicals were weighed on a Mettler AE160 electronic balance (0.1 mg sensitivity, Mettler Instrument Co., Hightstown, NJ, USA). Animals were weighed on a 700 series triple beam balance with a 2610 g capacity (Ohaus, Florham Park, NJ, USA)

B.7. Glassware

All glassware was rinsed with tap water and cleaned in a dishwasher (Miele electronic 675, Miele Electronics, F.R.G.) with a biodegradable Sparkleen solution (Fisher Scientific, Fairlawn, NJ, USA). For test tubes, further cleaning was accomplished by soaking tubes in a Sparkleen solution overnight followed by sonication (Ultra-sonic Cleaner, Mettler Instruments Co., Hightstown, NJ, USA) before the dishwasher wash. All glassware was dried at 250°C for 1 h in a mechanical convection oven (Model 28, Precision Scientific Group, Baxter Corp., Edmonton, AB, Canada).

B.8. Sample Pipettes

The pipettes used were one of three types: preset volume pipettes (10, 25, 50, 100 µl; Socorex, Switzerland), variable volume pipettes (20, 200, 1000 µl capacity; Gilson, France) or repeated volume pipettes (1.25, 5.0, 12.5, 50.0 ml capacity; Eppendorf, USA).

B.9. Shakers-Mixers

Mixing and vortexing of individual tubes and small conical flasks was performed on a benchtop Thermolyne Maxi Mix 1® (Sybron Corp.,

Dubuque, IA, USA). Large volume mixing with magnetic stir bars was done on either a benchtop Thermolyne Type 1000 (Sybron) or on a benchtop Thermix Model 210T mixer (Fisher Scientific, Fairlawn, NJ, USA).

C. ANIMALS

Male Sprague Dawley rats (Bioscience Animal Services, Ellerslie, AB, Canada) weighing 200-250 grams were purchased for this study. The animals were housed in pairs under a 12 hour light/dark cycle at a room temperature of 21±1°C. Food and water were freely available. Animals were fed Lab-Blox Feed (Wayne Feed Division, Continental Grain Co., Chicago, IL, USA).

C.1. Drug Administration

All doses of drugs administered to animals are expressed as mg/free base kg. Acute and chronic drug administration was performed *via* subcutaneous injection with a tuberculin 1 ml syringe equipped with a 27 G½" needle (Becton Dickinson, Closter, NJ, USA). All drugs were dissolved in either saline or double distilled water and injected in a volume of 1 ml/kg animal

weight. All control animals were injected with the corresponding drug vehicle and treatments were randomized. For the repeated intermittent injections, either (-)-nicotine or saline was administered daily (5 d/wk) for three weeks before the acute drug testing started. In the behavioral studies, one drug dose, counterbalanced with a vehicle injection, was tested each week (2 d/wk) allowing a 5 d wash-out period between drug administrations. The drugs were tested in the same animals using a repeated measures design and, in total, two groups of animals were used for the behavioral analysis. For the neurochemical experiments, drugs were administered to individual animals groups in a randomized fashion and three brain regions were removed from each animal.

C.2. Tissue Collection and Storage

At a predetermined time after the drug injection, animals were killed by instant guillotine decapitation. The brain was removed as quickly as possible and immersed in a beaker of ice-cold saline. Subsequent brain dissection was carried out on a saline-soaked filter paper on a bed of ice. The CNuc, NAcc and hippocampi of each brain were quickly dissected out [Greenshaw et al., 1989]. Individual tissues were promptly frozen on solid

carbon dioxide, and then stored at -80°C until the time of analysis.

D. ANALYTICAL METHODS

D.1. Analysis of DA, 5HT, 5HIAA, DOPAC and HVA Levels in Rat Brain Using the HPLC-ED

Separate brain regions were homogenized in microfuge tubes in a solution of ice-cold $HClO_4$ (0.1 M) containing EDTA (0.27 mM) and ascorbic acid (0.05 mM). The dilution volumes were chosen according to the regional concentrations of the neurochemicals: 20 vols for NAS, 5 vols for CN and 5 vols for HP. The homogenate was centrifuged on high setting for 2 min. Supernatant samples (150 µl) were stored in triplicate at -80°C until the time of analysis. Aliquots (15 µl) of each supernatant were injected onto the HPLC-ED system described in section B.1. A standard curve consisting of known concentrations of the neurotransmitters and their metabolites was run in parallel with the samples in order to allow identification of the peaks of interest and their quantification.

D.2. Locomotor Activity Measurements

Animal locomotion was recorded using the system described in section A.2. Animals were given their drug regimen and then monitored over a 30 min test period. The locomotor activity testing was performed in a separate room to reduce noise disturbances. Also, since rats are nocturnal, the room was lit by low-intensity lighting to simulate dark conditions. At the end of each test period, the animals were returned to their respective cages for the continuation of their drug regimen.

E. STATISTICAL ANALYSIS

All data were analyzed by one-, two- or three-way analysis of variance (ANOVA) followed by an independent t-test for single pair comparisons, or by a Newman-Keuls test for multiple comparisons. All probabilities are two-tailed. Statistical significance was based on the probability value of $p<0.05$. All behavioral results are based on eight animals per treatment group; neurochemical results are based on a minimum of five animals per group.

RESULTS

A. LOCOMOTOR ACTIVITY RESULTS

A.1. Effects of Chronic Nicotine Administration

The effect of chronic (-)-nicotine (0.30, 0.60, 1.20 mg/kg, sc daily, 16 d) on total locomotor activity is shown in Figure 7 as a percentage of baseline level for saline-treated rats. All three doses of nicotine tested significantly increased the total activity of rats. Locomotor activity counts in saline-treated control animals averaged 2076 ± 96 counts/30 min. Based on these results, a daily nicotine dose of 0.60 mg/kg (s.c, 21 d) was used in subsequent testing.

A.2. Effects of Acute Administration of $5HT_{1A}$ Receptor Ligands

The dose-response results for (±)-8-OHDPAT-induced (0.003, 0.030, 0.300 mg/kg sc, latency 15 min) changes in total locomotor activity counts for saline and nicotine-treated rats are shown in Figure 8. The drug significantly increased the total locomotor activity of saline-treated rats at 0.030 and 0.300 mg/kg, but did not significantly affect the behavior of nicotine-treated rats.

At 0.03 and 0.3 mg/kg,

8-OHDPAT increased the activity of the saline-treated rats 53% and 75%

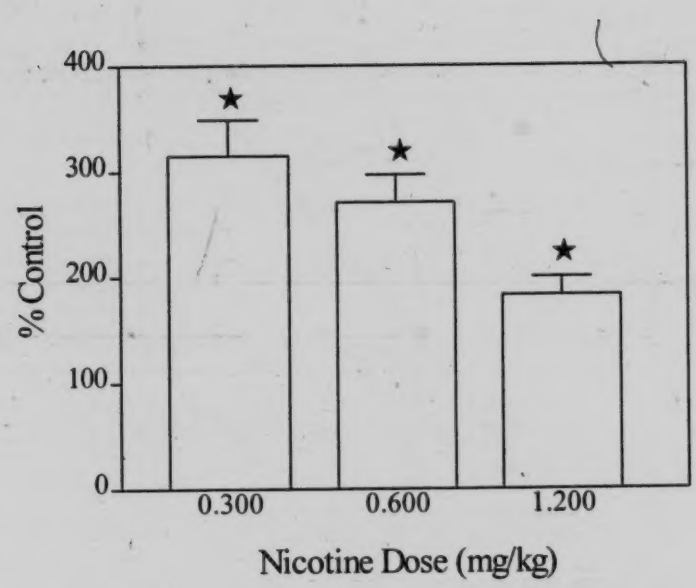


Figure 7: Dose response effects of 16 d repeated intermittent (-)-nicotine administration (mg/kg sc/d; n=8) on the spontaneous locomotor activity in the rat. Results expressed as percentage mean ± SEM of the total activity count for saline-treated rats (n=8). ⊤: SEM for each mean; ★ : significantly different from saline controls.

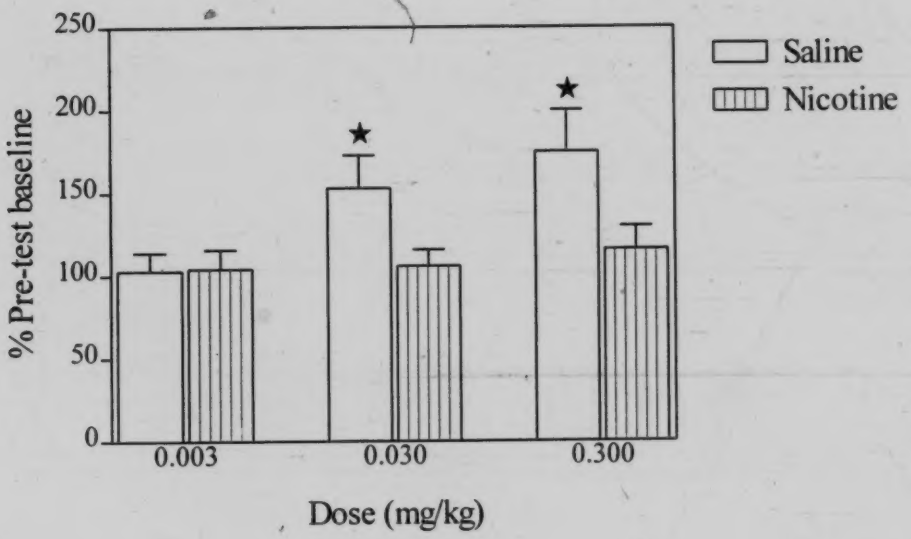


Figure 8: Dose response effects of acute 8-OHDPAT (mg/kg sc, latency 15 min, n=9) on the locomotor activity of (-)-nicotine- (0.6 mg/kg sc/d, 21d) and saline-treated rats. Results expressed as percentage mean ± SEM of the total activity count for pre-test baseline measured in saline-treated rats. ⊤: SEM for each mean; ★ : significantly different from other doses within each treatment group.

above baseline, respectively. The total locomotor activity of nicotine-treated rats was significantly reduced by acute treatment with NAN 190: to 73% of baseline at 0.30 mg/kg and to 69% of baseline at 3.0 mg/kg. The drug had no significant results in saline-treated animals. Figure 9 shows the NAN 190 (0.03, 0.30, 3.0 mg/kg sc, latency 15 min) dose-response results for total locomotor activity in saline- and nicotine-treated rats.

The $5HT_{1A}$ anatagonist, WAY 100635, did not significantly change the behavior of saline- or nicotine-treated rats at 0.1 mg/kg (sc, latency 15 min), as shown in Figure 11.

A.3. Effects of Acute Administration of $5HT_{1B}$ Receptor Ligands

The dose-response results for the $5HT_{1B}$ receptor agonist, TFMPP-induced (1.25, 2.50, 5.00 mg/kg sc, latency 10 min) changes of locomotor activity are shown in Figure 10. Acute treatment with all three doses (sc, latency 10 min) significantly and dose-dependently attenuated the total locomotion of nicotine-treated rats: to 71%, 56% and 46% of baseline values. Administration of 5.0 mg/kg also reduced the locomotor activity of saline-treated rats to 31% of basal levels. In subsequent testing, a dose of 3.0 mg/kg (sc, latency 15 min) TFMPP reduced the total activity of saline-treated rats

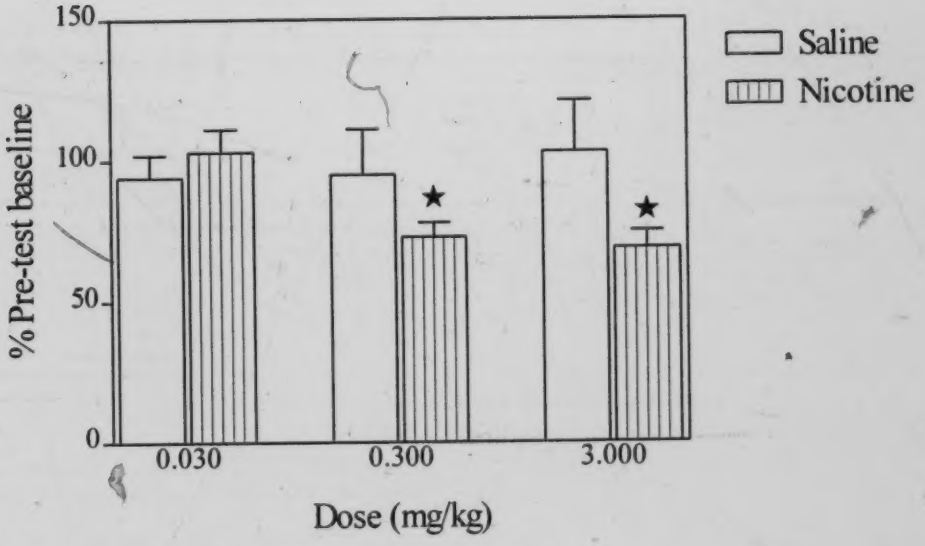


Figure 9: Dose response effects of acute NAN 190 (mg/kg sc, latency 15 min, n=9) on the locomotor activity of (-)-nicotine- (0.6 mg/kg sc/d, 21d) and saline-treated rats. Results expressed as percentage mean ± SEM of the total activity count for pre-test baseline measured in saline-treated rats. ⊤: SEM for each mean; ★ : significantly different from other doses within each treatment group.

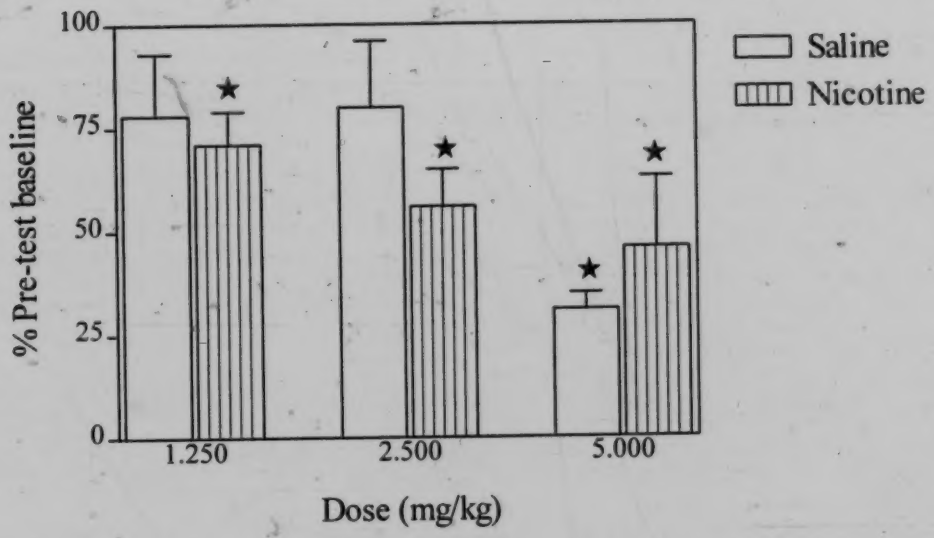


Figure 10: Dose response effects of acute TFMPP (mg/kg sc, latency 10 min, n=9) on the locomotor activity of (-)-nicotine- (0.6 mg/kg sc/d, 21d) and saline-treated rats. Results expressed as percentage mean ± SEM of the total activity count for pre-test baseline measured in saline-treated rats. ⊤: SEM for each mean; ★ : significantly different from other doses within each treatment group.

and of nicotine-treated rats to 49% and 46% of baseline, respectively (Figures 11 and 12).

Acute administration of RU 24969 (2.50 mg/kg sc, latency 10 min), another $5HT_{1B}$ agonist, significantly increased the total activity and rearing counts for both saline- and nicotine-treated rats, as shown in Figure 13. The activity of saline-treated rats was elevated by 295%, and that of nicotine-treated rats was elevated by 368%, above baseline levels. Dose-response testing was not completed for RU 24969 due to limited availability of the compound at the time of experimentation.

At a dose of 4.0 mg/kg, the non-selective $5HT_{1B}$ antagonist pindolol did not significantly alter the total locomotion of saline- or nicotine-treated animals (Figures 12 and 13).

A.4. Effects of Acute Administration of $5HT_2$ Receptor Ligands

In locomotor testing of the $5HT_2$ antagonist ritanserin, a dose of 5.0 mg/kg had no effect on the total activity of saline- or nicotine-treated rats.

A.5. Interaction of Acute Administration of $5HT_{1B}$ Receptor Agonist (TFMPP) and $5HT_{1A}$ Antagonist (WAY 100635)

The effect of coadministration of the $5HT_{1B}$ agonist TFMPP (3.0 mg/kg sc, latency 15 min) and the $5HT_{1A}$ antagonist WAY 100635 (0.10 mg/kg sc, latency 15 min) on locomotor activity is shown in Figure 11. WAY 100635 does not interact with TFMPP since it does not affect the TFMPP-induced hypoactivity in either chronic treatment group.

A.6. Interaction of Acute Administration of $5HT_{1B}$ Receptor Agonist (TFMPP) and Antagonist ((±)-Pindolol)

The effects of TFMPP (3.0 mg/kg sc, latency 15 min) and the $5HT_{1B}$ antagonist (±)-pindolol (4.0 mg/kg sc, latency 30 min) on total locomotor acitivity are shown in Figure 12. Pindolol does not significantly change the TFMPP-induced hypoactivity in either the saline- or nicotine-treated rats.

A.7. Interaction of Acute Administration of $5HT_{1B}$ Agonist (RU 24969) and Antagonist (Pindolol)

The effect of RU 24969 (2.5 mg/kg sc, latency 10 min) and pindolol (4.0

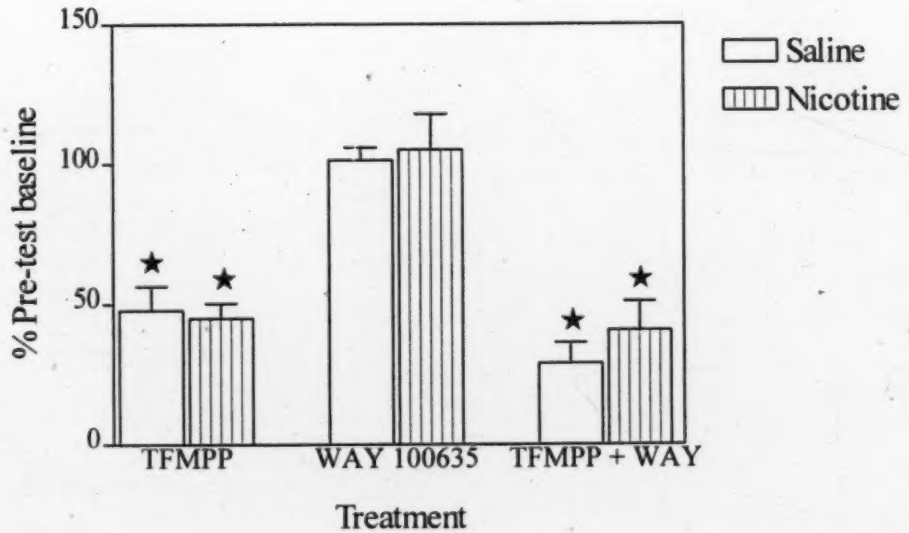


Figure 11: Effects of acute TFMPP (3.0 mg/kg sc, latency 10 min, n=8) and WAY 100635 (0.10 mg/kg sc, latency 15 min, n=8) on the locomotor activity of (-)-nicotine- (0.6 mg/kg sc/d, 21d) and saline-treated rats. Results expressed as percentage mean ± SEM of the total activity count for pre-test baseline measured in saline-treated rats. ⊤: SEM for each mean; ★ : significantly different from other doses within each treatment group.

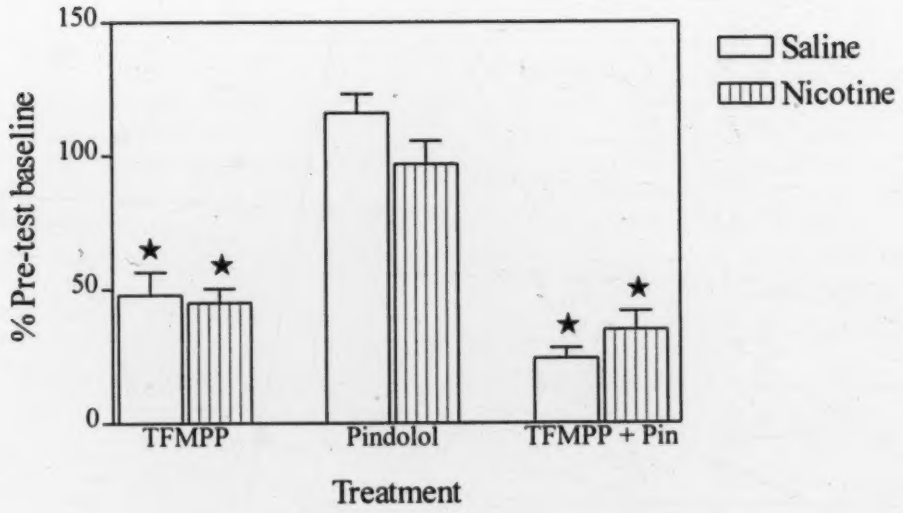


Figure 12: Effects of acute TFMPP (3.0 mg/kg sc, latency 10 min, n=8) and (±)-pindolol (4.0 mg/kg sc, latency 30 min, n=8) on the locomotor activity of (-)-nicotine- (0.6 mg/kg sc/d, 21d) and saline-treated rats. Results expressed as percentage mean ± SEM of the total activity count for pre-test baseline measured in saline-treated rats. ⊤: SEM for each mean; ★ : significantly different from other doses within each treatment group.

mg/kg sc, latency 30 min) on total locomotor activity is shown in Figure 13. Pindolol significantly attenuates the hyperactivity induced by acute RU 24969 in both saline- and nicotine-treated rats. The hyperactivity of saline-treated rats is reduced to 168% from 295%, and the hyperactivity of nicotine-treated rats is reduced to 210% from 368% of baseline.

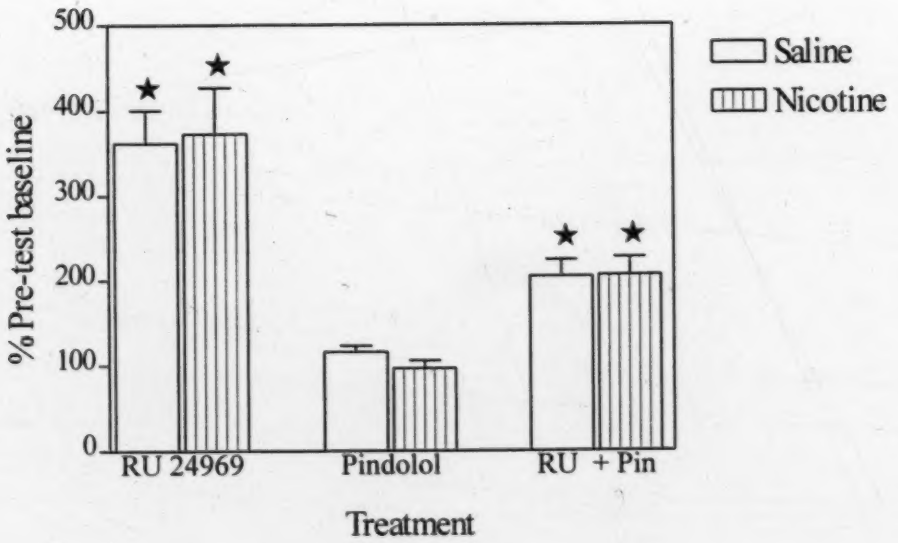


Figure 13: Effects of acute RU 24969 (2.5 mg/kg sc, latency 10 min, n=8) and (±)-pindolol (4.0 mg/kg sc, latency 30 min, n=8) on the locomotor activity of (-)-nicotine- (0.6 mg/kg sc/d, 21d) and saline-treated rats. Results expressed as percentage mean ± SEM of the total activity count for pre-test baseline measured in saline-treated rats. ⊤: SEM for each mean; ★ : significantly different from other doses within each treatment group.

B. NEUROCHEMICAL RESULTS

A sample liquid chromatograph obtained from the nucleus accumbens is shown in Figure 14.

B.1. Comparison of Neurochemical Levels of Saline- and Nicotine-Treated Rats to Previously Published Results

The neurochemical analysis of the brain regions in the present study was carried out using HPLC-ED. The baseline results for both saline- and nicotine-treated rats have been compared to results obtained by other groups using similar techniques as shown in Table 5.

B.2. Neurochemical Effects of Nicotine and 5HT Receptor-Related Ligands on the Hippocampus

The neurochemical effects elicited by these compounds in the hippocampus are shown in Figure 15.

Chronic (-)-nicotine (0.60 mg/kg sc, 21 d) significantly increased the baseline DA levels in the hippocampus compared to chronic saline control animals.

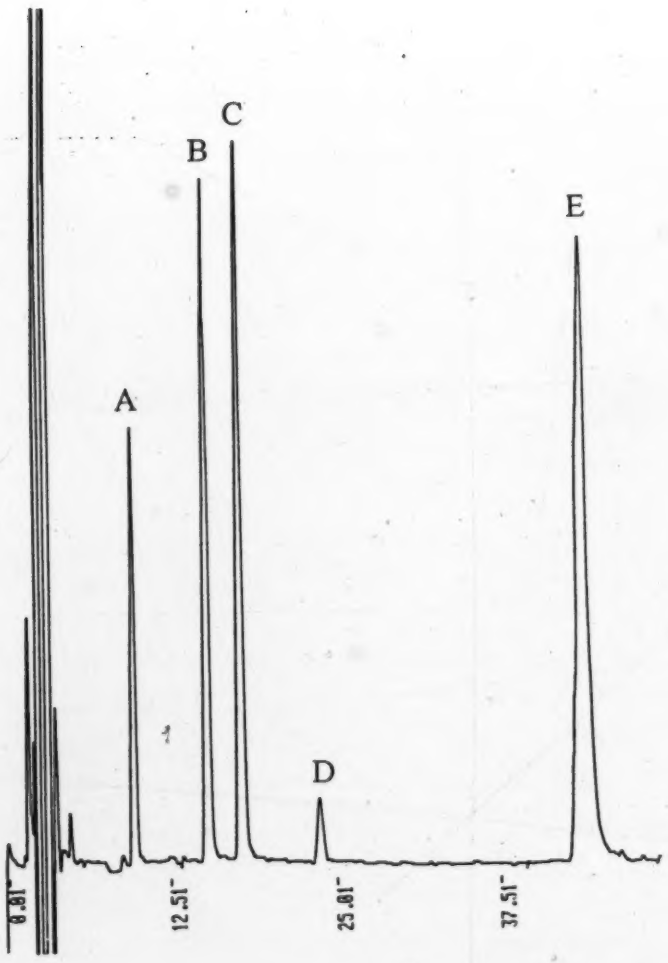


Figure 14: Typical liquid chromatograph obatined from the extract of a rat nucleus accumbens sample. Legend: DOPAC (A), DA(B), 5HIAA (C), HVA (D), 5HT (E).

Table 5: Comparison of HPLC-ED Results to Similar Studies:

Chronic Saline and Nicotine Treatment of the Rat
(results expressed as ng/g tissue)

Neurotransmitter or Metabolite		SALINE				NICOTINE	
		Present Results	Roberts	Lapin	Mousseau	Present Results	Lapin
Nucleus Accumbens	DA	4900	1825	7923	-	4052	1443
	DOPAC	4881	1007	2502	-	4969	5322
	HVA	1526	234	1104	-	1448	552
	5HT	387	683	775	-	280	550
	5HIAA	621	640	598	-	603	380
Caudate Nucleus	DA	4309	8032	13000	8200	3940	12280
	DOPAC	3100	2111	2759	3000	2821	2257
	HVA	909	780	1592	850	847	1202
	5HT	236	559	288	490	232	260
	5HIAA	398	500	368	710	400	357
Hippocampus	DA	29	17	-	30	-	-
	DOPAC	10	17	-	35	-	-
	HVA	5	-	-	20	-	-
	5HT	244	149	-	240	-	-
	5HIAA	368	327	-	600	-	-

[Lapin et al., 1989; Mousseau, 1991; Roberts et al., 1994]

The other neurochemical levels were not affected.

Acute administration of the $5HT_{1A}$ receptor agonist, 8-OHDPAT (0.30 mg/kg sc, latency 15 min) increased DA levels in saline-treated rats, and NAN 190 (5.00 mg/kg sc, latency 15 min) significantly reduced DA levels in both chronically treated groups. The HVA levels in nicotine-treated rats with acute 8-OHDPAT administration were below the limits of detection.

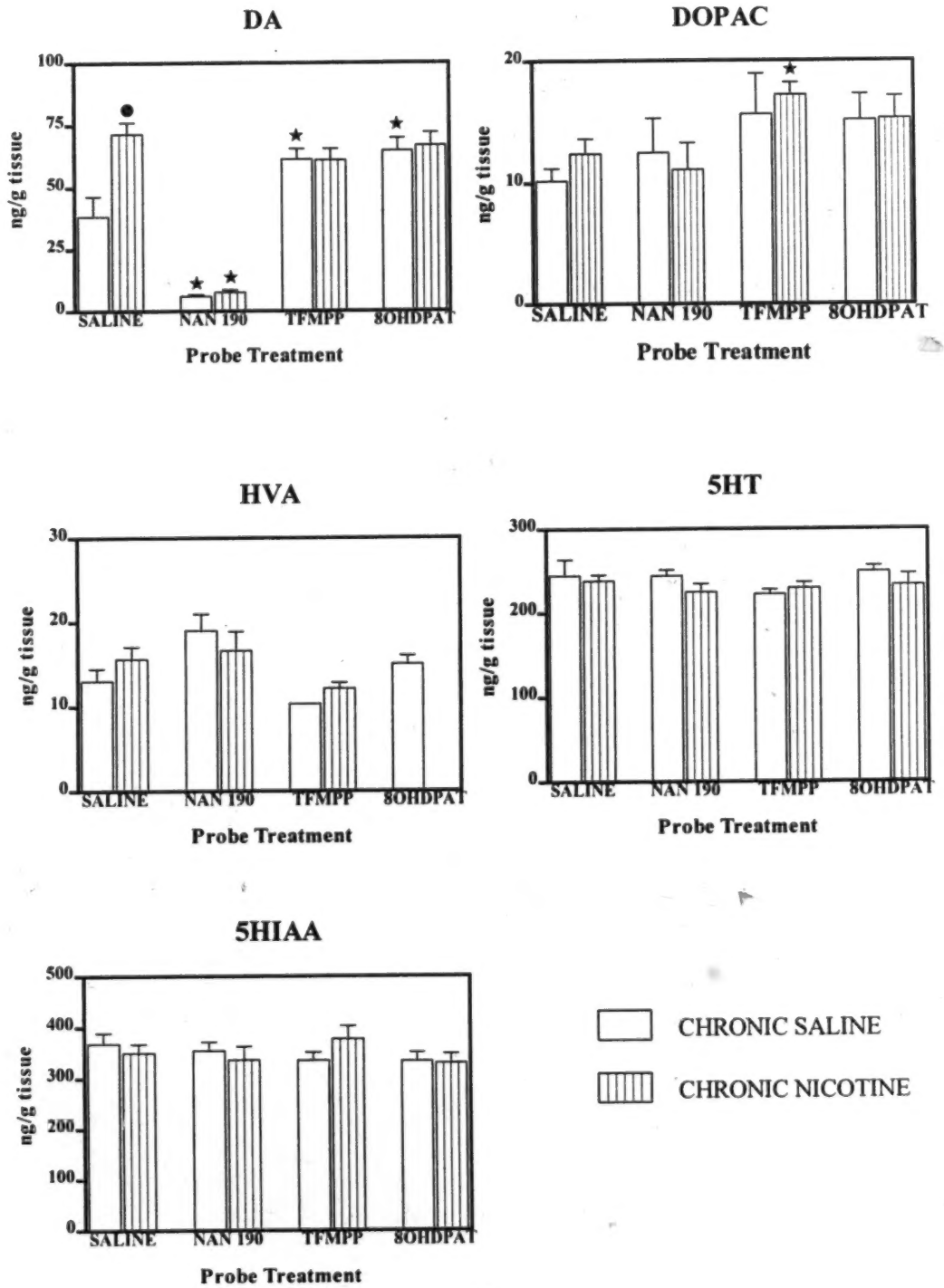
The $5HT_{1B}$ receptor agonist, TFMPP (3.0 mg/kg sc, latency 10 min), significantly increased hippocampal DA levels in saline-treated rats. The nicotine-treated animals also had significantly elevated DOPAC in the hippocampus following acute administration of this drug.

B.3. Neurochemical Effects of Nicotine and 5HT Receptor-Related Ligands on the Caudate Nucleus

The neurochemical effects elicited by these compounds in the hippocampus are shown in Figure 16.

Chronic (-)-nicotine (0.60 mg/kg sc, 21 d) did not have a significant effect on

Figure 15: Effect of acute administration of TFMPP (3.0 mg/kg sc, latency 10 min, n=8), 8-OHDPAT (0.3 mg/kg sc, latency 15 min, n=8) or NAN 190 (5.00 mg/kg sc, latency 15 min, n=6) on the tissue levels of DA, DOPAC, HVA, 5HT and 5HIAA in the hippocampus of saline- and nicotine-treated (0.6 mg/kg sc, 21d) rats. Results are expressed as mean ± SEM. • = significantly different from saline-treated vehicle group; ★ = significantly different from other treatments within the same group.


DA
ng/g tissue
100
75
50
25
0
SALINE
NAN 190
TFMPP
8OHDPAT
Probe Treatment
DOPAC
ng/g tissue
20
10
0
SALINE
NAN 190
TFMPP
8OHDPAT
Probe Treatment
HVA
ng/g tissue
30
20
10
0
SALINE
NAN 190
TFMPP
8OHDPAT
Probe Treatment
5HT
ng/g tissue
300
200
100
0
SALINE
NAN 190
TFMPP
8OHDPAT
Probe Treatment
5HIAA
ng/g tissue
500
400
300
200
100
0
SALINE
NAN 190
TFMPP
8OHDPAT
Probe Treatment
CHRONIC SALINE
CHRONIC NICOTINE

the caudate nucleus DA, DOPAC, HVA, 5HT or 5HIAA levels compared to chronic saline.

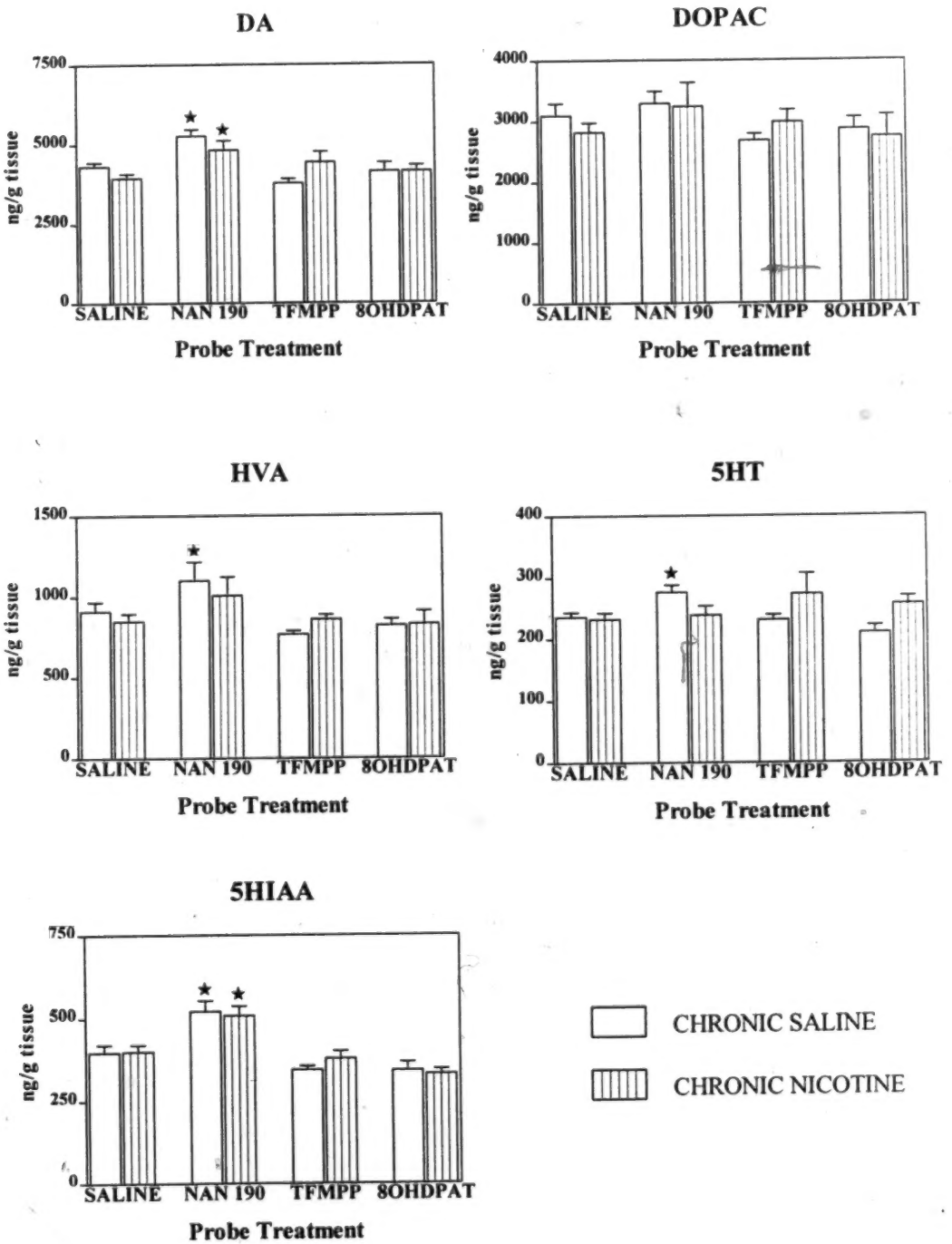
Of the $5HT_{1A}$ receptor agonists, only NAN 190 (5.0 mg/kg sc, latency 15 min) had effects in the caudate nucleus: it significantly increased DA, HVA, 5HT and 5HIAA levels in saline-treated rats, and elevated DA and 5HIAA levels in nicotine-treated rats. 8-OHDPAT (0.30 mg/kg sc, latency 15 min) administration did not alter the neurochemistry of the caudate nucleus.

Acute administration of the $5HT_{1B}$ receptor agonist TFMPP (3.00 mg/kg, latency 15 min) also did not significantly alter the levels of the neurotransmitters or their metabolites in this brain region.

B.4. Neurochemical Effects of Nicotine and 5HT Receptor-Related Ligands on the Nucleus Accumbens

The neurochemical effects elicited by these compounds in the hippocampus are shown in Figure 17.

Figure 16: Effect of acute administration of TFMPP (3.0 mg/kg sc, latency 10 min, n=8), 8-OHDPAT (0.3 mg/kg sc, latency 15 min, n=8) or NAN 190 (5.00 mg/kg sc, latency 15 min, n=6) on the tissue levels of DA, DOPAC, HVA, 5HT and 5HIAA in the caudate nucleus of saline- and nicotine-treated (0.6 mg/kg sc, 21d) rats. Results are expressed as mean ± SEM. ★ = significantly different from other treatments within the same group.

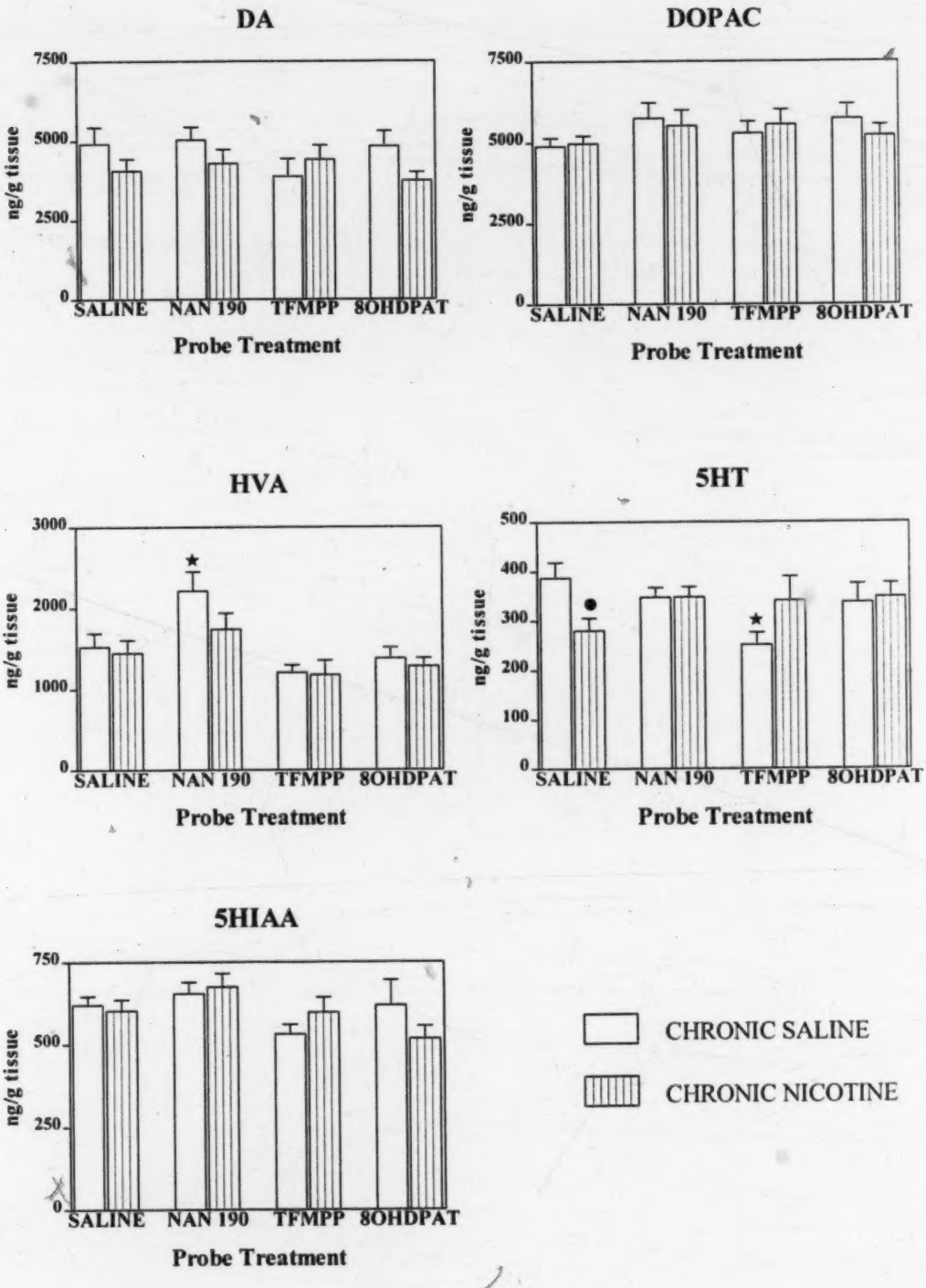

DA
7500
5000
2500
0
ng/g tissue
SALINE
NAN 190
TFMPP
8OHDPAT
Probe Treatment
DOPAC
4000
3000
2000
1000
0
ng/g tissue
SALINE
NAN 190
TFMPP
8OHDPAT
Probe Treatment
HVA
1500
1000
500
0
ng/g tissue
SALINE
NAN 190
TFMPP
8OHDPAT
Probe Treatment
5HT
400
300
200
100
0
ng/g tissue
SALINE
NAN 190
TFMPP
8OHDPAT
Probe Treatment
5HIAA
750
500
250
0
ng/g tissue
SALINE
NAN 190
TFMPP
8OHDPAT
Probe Treatment
CHRONIC SALINE
CHRONIC NICOTINE

Chronic (-)-nicotine (0.60 mg/kg sc, 21 d) significantly decreased 5HT levels, but did not significantly affect the levels of DA or its metabolites, compared to chronic saline control animals.

There were minimal changes elicited by $5HT_{1A}$ receptor agonists in the nucleus accumbens. In saline-treated animals, NAN 190 (5.0 mg/kg sc, latency 15 min) significantly increased the HVA levels. 8-OHDPAT (0.30 mg/kg sc, latency 15 min) did not have any significant effects on neurotransmitter levels.

Acute administration of TFMPP (3.0 mg/kg, latency 15 min) significantly decreased the 5HT level in the nucleus accumbens of saline-treated rats.

Figure 17: Effect of acute administration of TFMPP (3.0 mg/kg sc, latency 10 min, n=8), 8-OHDPAT (0.3 mg/kg sc, latency 15 min, n=8) or NAN 190 (5.00 mg/kg sc, latency 15 min, n=6) on the tissue levels of DA, DOPAC, HVA, 5HT and 5HIAA in the nucleus accumbens of saline- and nicotine-treated (0.6 mg/kg sc, 21d) rats. Results are expressed as mean ± SEM. • = significantly different from saline-treated vehicle group; ★ = significantly different from other treatments within the same group.


DA
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NAN 190
TFMPP
8OHDPAT
Probe Treatment
DOPAC
7500
5000
2500
0
ng/g tissue
SALINE
NAN 190
TFMPP
8OHDPAT
Probe Treatment
HVA
3000
2000
1000
0
ng/g tissue
SALINE
NAN 190
TFMPP
8OHDPAT
Probe Treatment
5HT
500
400
300
200
100
0
ng/g tissue
SALINE
NAN 190
TFMPP
8OHDPAT
Probe Treatment
5HIAA
750
500
250
0
ng/g tissue
SALINE
NAN 190
TFMPP
8OHDPAT
Probe Treatment
CHRONIC SALINE
CHRONIC NICOTINE

DISCUSSION

A. BEHAVIORAL EFFECTS OF NICOTINE AND 5HT RECEPTOR LIGANDS

A.1. Nicotine

In the current study, an unique examination of the effects of specific 5HT receptors on the DA activity of basal and activated mesolimbic pathways was completed. The working hypothesis for this study was that 5HT has an inhibitory effect on midbrain DA activity. Therefore receptor effects which result in a decreased 5HT release were expected to disinhibit DA release in the NAcc and locomotor activity. In a novel approach, the neurochemical and behavioral effects of $5HT_{1A}$, $5HT_{1B}$ and $5HT_2$ receptor compounds in this case were measured in rats using the nicotine hyperactivity model. Based on this model, intermittent nicotine treatment will induce hyperactivity in rats and, in the present study, the systemic daily administration of nicotine did significantly increase the locomotor activity of rats in a dose-dependent manner.

A.2. $5HT_{1A}$ Receptor Ligands

The role of the $5HT_{1A}$ receptor in regulating mesolimbic DA was tested using a putative agonist (8-OHDPAT), partial agonist (NAN 190), and selective antagonist (WAY 100635). This receptor subtype exists primarily as an autoreceptor on raphé nuclei, including the DRN, although it has also been identified as a DA heteroreceptor and postsynaptically in the hippocampus [Boess and Martin, 1994]. Stimulation of the $5HT_{1A}$ autoreceptor is hypothesized to decrease 5HT release and yield an increase in the DA tone and locomotor activity.

In support of this $5HT_{1A}$ receptor stimulation hypothesis, 8-OHDPAT significantly increased the spontaneous locomotor activity (saline-treated) of rats in the present study. This would be consistent with a release DA inhibition by 5HT and may be due to 8-OHDPAT-mediated feedback inhibition at $5HT_{1A}$ autoreceptors in the DRN. There was no significant increase in the behavior of hyperactive (nicotine-treated) rats. This may be related to a ceiling hyperactivity level in sensitized rats which may override the increased DA release induced by 8-OHDPAT. In contrast, NAN 190, also classified as a $5HT_{1A}$ agonist, significantly decreased the activity of saline- and nicotine-

treated rats. This discrepancy indicates that NAN 190 likely acts by a different mechanism than 8-OHDPAT in this behavioral model and questions the functional equivalence of these two compounds.

It is notable that 8-OHDPAT and NAN 190 are both classified as partial $5HT_{1A}$ receptor agonists, but there is evidence which suggests that they are functionally different, as was found in the current study, and some theories to explain these data. One suggestion is that the two compounds may have different effects on presynaptic and postsynaptic receptors which would contribute to their observed functional differences. The two compounds have unique pharmacological profiles based on their affinities for other receptors: NAN 190 is an antagonist at α_1 receptors and 8-OHDPAT has an affinity for $5HT_7$ receptors [Glennon et al., 1988; Kawahara et al., 1994; Middlemiss and Hutson, 1992; Tsou et al., 1994]. Although the $5HT_7$ receptor subtype has not been implicated in the regulation of locomotor activity, the α_1 receptor antagonist, prazosin, inhibits the locomotor activity induced by intra-NAcc (+)-amphetamine administration [Blanc et al., 1994]. This suggests that α_1 receptor binding properties of NAN 190 may be responsible for the observed differences in behavior compared to 8-OHDPAT. The present data are in

agreement with the differential behavioral profiles of NAN 190 and 8-OHDPAT in other drug comparison models, such as drug discrimination learning studies [Glennon et al., 1988; Schreiber and de Vry, 1993; Schreiber et al., 1995]. 8-OHDPAT discrimination learning, where animals are trained to discriminate between the stimulus properties of 8-OHDPAT and saline, have been completed and NAN 190 is found to block, not mimic, the 8-OHDPAT cue [Glennon et al., 1988; Schreiber and de Vry, 1993; Screiber et al., 1995]. In contrast, NAN 190 and 8-OHDPAT showed the same pattern of results in electrical VTA self-stimulation studies, another useful comparative measure of the effects of 5HT receptor-related compounds on the activity of the mesolimbic DA system [Greenshaw, 1995]. These studies appear to support the proposed differences in the mechanisms of the putative $5HT_{1A}$ receptor agonists, NAN 190 and 8-OHDPAT, however further indepth studies will be necessary to clarify their observed behavioral differences.

Administration of the selective $5HT_{1A}$ antagonist, WAY 100635, did not alter spontaneous or nicotine-induced hyperactivity in rats. Primarily, this suggests that tonic $5HT_{1A}$ autoreceptor feedback may not be a factor in the proposed 5HT inhibition of DA release. Nevertheless, if the $5HT_{1A}$ receptor-mediated

change in locomotor activity effect is a site-specific phenomenon, then two other explanations may be considered. If the $5HT_{1A}$ receptor-mediated behavior is regulated at presynaptic sites then blocking $5HT_{1A}$ autoreceptors with a silent antagonist, such as WAY 100635, would prevent endogenous 5HT feedback inhibition. This may result in an increase in 5HT release and therefore, according to the working hypothesis, would potentiate existing 5HT inhibition of DA activity. Alternatively, if a tonic feedback inhibition does not exist then WAY 100635 will not alter the 5HT release and a corresponding change would not be expected in the activity of the DA system. However, if the locomotor activity is due to interaction with postsynaptic $5HT_{1A}$ receptors, then WAY 100635 given alone might limit the expression of locomotor activity *via* stimulation of these receptors. The current studies provide useful, preliminary information about the role of the $5HT_{1A}$ receptor in regulating locomotor behavior and encourage further behavioral testing with $5HT_{1A}$ receptor-related compounds.

A.3. $5HT_{1B}$ Receptor Ligands

In view of the limited availability of drugs selective for this receptor subtype, ligands with a relatively high $5HT_{1B}$ receptor affinity were used to analyze the

effect of $5HT_{1B}$ receptors in DA mesolimbic control in this study. The $5HT_{1B}$ receptor has been identified as a terminal autoreceptor on DRN projections, and it also functions as a heteroreceptor on mesolimbic DA terminals and as a postsynaptic receptor [Boess and Martin, 1994; Middlemiss and Hutson, 1990]. According to the current hypothesis, stimulation of $5HT_{1B}$ autoreceptors will decrease 5HT release and promote hyperactivity.

As expected, the more selective $5HT_{1B}$ agonist, RU 24969 induced hyperlocomotion in saline- and nicotine-treated rats. In addition, this hyperactivity was attenuated by co-administration of the $5HT_{1B}$ antagonist, (±)-pindolol. These results lend strong support to the hypothesis of 5HT inhibition of DA activity, and indicate that it may be regulated, in part, by $5HT_{1B}$ autoreceptor feedback. Attributing the behavioral effects of RU 24969 to its action at other sites, namely the $5HT_{1A}$, DA and α_1 receptors, cannot be ruled out since (±)-pindolol has $5HT_{1A}$ receptor antagonist properties and the RU 24969-induced behavior is dose-dependently blocked by non-selective DA and α_1 receptor antagonists, including haloperidol, and by the selective $5HT_{1A}$ antagonist, WAY 100635 [Kalkman, 1995; Matos et al., 1996; Tricklebank et al., 1986]. Notably, the α_1 receptor agonists phenylephrine and methoxamine

have locomotor activating effects which are also blocked by haloperidol, which further supports a role of α_1 receptors in locomotion [Tricklebank et al, 1986]. However, in the current experimental model, RU 24969 and 8-OHDPAT did not demonstrate the same pattern of locomotor activity which suggests that RU 24969 is likely not acting at $5HT_{1A}$ receptors. (±)-Pindolol administered alone did not significantly affect the behavior of rats in either chronic treatment group.

Unlike RU 24969, the purported $5HT_{1B}$ agonist TFMPP reduced the activity of saline- and nicotine-treated rats. This inhibitory effect on spontaneous and activated rats by TFMPP suggests that it is acting by a different mechanisms than RU 24969. Also, unlike RU 24969, pindolol did not prevent the TFMPP-induced locomotor changes. Furthermore, this hypoactivity was not blocked by the $5HT_{1A}$ antagonist, WAY 100635. So, the behavioral actions of TFMPP in this hyperactivity model are likely mediated by a different mechanism that RU 24969.

Data from other behavioral studies lend support to the observed functional differences of the $5HT_{1B}$ receptor agonists, TFMPP and RU 24969. Additional

locomotor activity blocking studies suggest that the unique locomotor suppressing effects of TFMPP may be due to its interactions with non-$5HT_{1A}$ and $5HT_{1B}$ receptors, such as the $5HT_{2C}$ receptor which is believed to play a role in the regulation of locomotor activity [Krebs and Geyer, 1994; Lucki et al., 1989; Motta et al., 1992]. Specifically, the decreased locomotor activity effects of ketanserin, a $5HT_{2A/2C}$ receptor antagonist and d-lysergic acid diethylamide (LSD), a non-specific $5HT_1$ and $5HT_2$ receptor agonist, are believed to be mediated, in part, by the $5HT_{2A/2C}$ receptor subtypes [Krebs and Geyer, 1994; Motta et al., 1992]. The affinity of TFMPP for the $5HT_{2C}$ receptor is an important distinction in its pharmacological profile compared to that of RU 24969 and may be a factor in their different behavioral effects. Structurally, the two compounds are also different [Middlemiss and Hutson, 1990; Tricklebank et al., 1986] which would affect their receptor interactions and may help explain their observed effects. In contrast, TFMPP drug discrimination and ICSS studies do not differentiate between the TFMPP and RU 24969 behavioral effects [Arnt, 1989; Cunningham and Appel, 1986; Greenshaw, 1995; Swedberg et al., 1992]. In ICSS studies, the effects of TFMPP are potentiated by coadministration with (±)-pindolol [Greenshaw, 1995], whereas an interaction was not observed using these two compounds in

the present hyperactivity model. Nevertheless, based on this data the current classification of RU 24969 and TFMPP as $5HT_{1B}$ receptor agonists proves inadequate for the present behavioral model and, as with the $5HT_{1A}$ receptor agonists, further functional analysis is required.

A.4. $5HT_2$ Receptor Ligands

$5HT_2$ receptors are located on DA neurons in the VTA and at other postsynaptic sites in the brain [Andersson et al, 1995; Brodie and Bunney, 1996]. Based on the above hypothesis, blocking these receptors would prevent 5HT inhibition of mesolimbic DA activity and yield hyperactivity. In the current experiment, the $5HT_2$ receptor antagonist ritanserin did not alter the locomotor activity of saline- or nicotine-treated rats at a dose that is reported to have significant effects in other behavioral studies, including locomotor activity and exploratory activity tests [Middlemiss and Tricklebank, 1992; Peltier et al., 1994], so this receptor subtype does not appear to mediate the inhibition of 5HT on DA neurons.

B. NEUROCHEMICAL EFFECTS OF NICOTINE AND 5HT RECEPTOR LIGANDS

In conjunction with behavioral testing, a neurochemical analysis of the 5HT receptor ligands was completed in three mesolimbic brain regions: the hippocampus, NAcc and CNuc. Again, these experiments were done on rats with spontaneous (repeated intermittent injections of saline-treated) and activated (repeated intermittent injections of nicotine-treated) mesolimbic pathways. The present results are in agreement with values reported in the literature for saline- and nicotine-treated rats. The wide range of values which is consistently recorded may be accounted for, in part, by differences in dissection procedures or HPLC-ED set up. These results were obtained following acute administration of the $5HT_{1A}$ receptor agonists, 8-OHDPAT and NAN 190, and the $5HT_{1B}$ agonist TFMPP. The goal was to link the behavioral changes to neurochemical alterations. Measurements of the DA and 5HT metabolites (DOPAC, HVA and 5HIAA) were also recorded in an attempt to identify metabolic changes which may be linked to these receptors sites. For example, increased DOPAC or HVA levels and static DA levels might correspond to an increased regional turnover of DA. Many 5HT receptor-related compounds have been shown to have effects on 5HT or DA turnover

and synthesis [Oberlander et al., 1987; Invernizzi et al., 1991; Middlemiss and Hutson, 1990].

Based on the current hypotheses of $5HT_{1A}$ and $5HT_{1B}$ receptors functioning as autoreceptors, a decrease in 5HT release and an increase in DA release and locomotor activity is expected following stimulation of these 5HT receptor subtypes. Neurochemically, this might be postulated to correspond to a decrease in 5HT and to an increase in DA. The present neurochemical results, however, were obtained by *ex vivo* analysis of whole tissue regions and therefore may not reflect the actual extracellular alterations. Nevertheless, these results are an important preliminary step in determining the role of 5HT receptors in mediating mesolimbic DA activity and they should be developed with *in vivo* microdialysis studies to identify the extracellular nature of these receptor effects.

B.1. Nicotine

In the present experiment, daily administration of nicotine resulted in the following significant neurochemical effects compared to saline controls: DA levels were increased in the hippocampus and 5HT levels were decreased in

the NAcc. In this study, the expected increase in NAcc DA was not observed. This may be function of the limitations of the *ex vivo* analysis techniques employed since previous *in vivo* microdialysis studies have shown that a similar nicotine regime does result in an increased NAcc DA release [Benwell and Balfour, 1992; Nisell et al., 1996].

B.2. $5HT_{1A}$ Receptor Ligands

As previously described, the $5HT_{1A}$ receptor functions presynaptically as an autoreceptor in the DRN and as a heteroreceptor on mesolimbic DA terminals, in addition to postsynaptically in the hippocampus [Boess and Martin, 1994]. Stimulation of $5HT_{1A}$ receptors should decrease 5HT release and disinhibit the DA system according to the working hypothesis of this thesis. In the present study, acute systemic administration of the $5HT_{1A}$ receptor agonist 8-OHDPAT (0.3 mg/kg s.c., 15 min latency) significantly increased DA levels in the hippocampus of the repeated intermittent injections of saline group, and had no effect in the CNuc or the NAcc of saline- or nicotine-treated rats, or on 5HT levels. The increase in terminal DA levels (hippocampus) following $5HT_{1A}$ receptor stimulation is in agreement with the hypothesized 5HT inhibition of DA. As with its locomotor activating effects, 8-OHDPAT was only observed

to have effects in saline-treated rats, but a corresponding increase in NAcc DA is not seen. Again, it is important to consider that the actual extracellular neurochemical changes induced by 8-OHDPAT may not be accurately reflected in these *ex vivo* measurements of whole tissue levels. In support of the present results for DA, studies have also shown that 8-OHDPAT does not affect the extracellular DA levels of the NAcc or CNuc [Arborelius et al., 1993; Jiang et al., 1990; Nomikos et al., 1996], however, conflicting results suggest that this effect might be a function of the dose or route of administration [Arborelius et al., 1993; Bencoulif et al., 1993; Cornfield et al., 1991]. Other studies also indicate that 8-OHDPAT may alter DA turnover in the hippocampus and striatum [Arborelius et al., 1993; Gobert et al., 1995; Matos et al., 1996], although there is no evidence of this in the present results.

Furthermore, the absence of significant 8-OHDPAT-induced changes in 5HT activity observed here underscores the limitations of the analysis techniques used since comparably, *in vivo* microdialysis studies demonstrate extracellular changes in 5HT release and turnover. Specifically, microdialysis shows a decrease in 5HT release in the hippocampus and striatum, which is blocked by $5HT_{1A}$ receptor antagonists WAY 100635 and (S)-UH-301 [Assie and Koek,

1996a; Fletcher et al., 1995; Ge and Barnes, 1996; Matos et al., 1996; Mundey et al., 1996; Nomikos et al., 1992; Saito et al., 1996], and a decrease in 5HT release from the DRN [Gobert et al., 1995; Millan et al., 1995]. The role of $5HT_{1A}$ receptors in regulating 5HT release appears to be site-specific since 8-OHDPAT administration in the DRN and MRN only reduced the 5HT release in the hippocampus and the striatum, respectively [Kreiss and Lucki, 1994]. 8-OHDPAT also attenuates 5HT turnover in the striatum and NAcc as shown by reduced 5HIAA microdialysate levels in these areas [Invernizzi et al., 1991; Nomikos et al., 1992].

Unlike the DA-limited effects of 8-OHDPAT on the rat brain, the $5HT_{1A}$ receptor agonist NAN 190 had significant effects on DA, 5HT and their metabolites in saline- and nicotine-treated rats. In the present study, DA levels were increased and decreased, respectively, in the CNuc nucleus and hippocampus of saline- and nicotine-treated rats following NAN 190. In addition, HVA levels were elevated in the caudate nucleus and nucleus accumbens of repeated intermittent injections of saline-treated rats only. Significant serotonergic effects due to NAN 190 were observed in the CNuc where 5HT levels were higher in the saline group, and 5HIAA levels were

significantly elevated in the saline and nicotine animals. The NAN 190-induced increase in CNuc DA release also supports the working hypotheses of 5HT inhibition of DA activity. Otherwise, the results from the study with NAN 190 appear to contradict the expected increase in DA release and decrease in 5HT release. However, these elevated 5HT and 5HIAA levels may be a function of decreased 5HT release resulting in the buildup and metabolism of excess 5HT in the presynaptic neuron. Otherwise, previous studies show that NAN 190 dose-dependently decreased 5HT turnover in the striatum and hippocampus [Gobert et al., 1995]. Despite similar radioligand binding profiles, the differential neurochemical and behavioral profiles of NAN 190 and 8-OHDPAT in this study may point to important differences in their pharmacological actions [Rydelek-Fitzgerald et al., 1990].

B.3. $5HT_{1B}$ Receptor Ligands

A final neurochemical analysis of the role of the $5HT_{1B}$ receptor in mesolimbic DA control was completed using TFMPP. In the hippocampus, the $5HT_{1B}$ receptor agonist significantly increased the DA levels of saline-treated rats and the DOPAC levels of nicotine-treated rats. In the NAcc, TFMPP reduced the 5HT levels in saline-treated animals. The TFMPP-induced neurochemical

changes in DA and 5HT levels appear to agree with the hypothesized role of $5HT_{1B}$ autoreceptor stimulation in the regulation of DA activity. Limited effects on the nicotine-treated rats may indicate that nicotine masks the TFMPP-induced alterations in these animals, and potentially help explain the discrepancy from the behavioral results. In other studies, using *in vivo* microdialysis, a decrease in 5HT release is recorded from the frontal cortex and hippocampus following TFMPP administration, similar to the 5HT decrease seen in the NAcc in the present study [Saito et al., 1996]. However, in contrast to these microdialysis findings, *in vitro* studies show increases in hippocampal 5HT release [Saito et al., 1996]. In addition, *in vitro* studies also record TFMPP inhibition of 5HT synthesis, likely through a suggested $5HT_{1B}$ receptor-mediated mechanism, which may contribute to the decreased NAcc 5HT levels recorded here [Hjorth et al., 1996]. In the present $5HT_{1B}$ receptor studies, TFMPP was chosen as the agonist for initial neurochemical and behavioral tests and a second compound, the novel $5HT_{1B}$ receptor agonist RU 24969, was subsequently used for behavioral testing only. The extracellular neurochemical effects of $5HT_{1B}$ receptor agonists, including TFMPP, and the receptors which mediate these changes should be further investigated in specific brain regions using *in vivo* microdialysis procedures. Nevertheless,

these neurochemical results suggest that the $5HT_{1B}$ receptor might play a role in mediating the 5HT inhibitory control of the mesolimbic DA system.

SUMMARY

In this study, for the first time an attempt was made to compare the effects of 5HT receptor-related compounds in the same behavioral model and to compare their neurochemical effects in specific brain regions. Initially, an hypothesis of 5HT inhibition of DA activity was proposed. Given the complexity of 5HT pharmacology, this hypothesis was an oversimplification but it provided a convenient approach to investigate 5HT-DA interactions. In a novel approach, these 5HT receptor-related compounds were tested in the same animals using a nicotine-hyperactivity model. The pattern of results obtained show significant differences between drugs which have been classified as ligands with a similar mechanism of action. This suggests a difference in the pharmacological profile of these compounds, particularly their receptor interactions, which may be a function of the animal model. Specifically, the proposed $5HT_{1A}$ receptor agonists, 8-OHDPAT and NAN 190, and $5HT_{1B}$ receptor agonists, TFMPP and RU 24969, were found to have significantly different neurochemical and behavioral effects in the nicotine-hyperactivity model.

The results support the proposal that 5HT exerts an inhibitory effect on the

activity of mesolimbic DA systems. For example, studies with the novel, selective $5HT_{1A}$ receptor antagonist, WAY 100635, demonstrate that there is likely no tonic action of endogenous 5HT at somatodendritic sites in the DRN and suggest that the action of the $5HT_{1A}$ receptor agonist, 8-OHDPAT, may be mediated at postsynaptic sites. Equally, since the $5HT_{1B}$ receptor agonist TFMPP is not blocked by the $5HT_{1B}$ receptor antagonist (±)-pindolol nor WAY 100635, these results are in agreement with previous studies which indicate that it may function *via* $5HT_{2C}$ receptors [Lucki et al., 1989]. In contrast, the more selective $5HT_{1B}$ receptor agonist, RU 24969, is blocked by (±)-pindolol. The $5HT_2$ receptor antagonist, ritanserin, failed to have an effect in the current behavioral model. However, this may be a consequence of the dose used, even though it was selected based on results from previous studies [Middlemiss and Tricklebank, 1992], and therefore a complete dose response analysis of ritanserin in this model should be completed.

The current *ex vivo* analysis using a nicotine-hyperactivity behavioral model is a useful preliminary step in the comprehensive analysis required to fully characterize these serotonergic drugs. Conclusions drawn from the this data provide direction and aim for future studies on these 5HT receptor-related

compounds. Specifically, these studies should include *in vivo* techniques which can better pinpoint the site of action and the receptor subtype involved, including microdialysis, voltammetry, radioligand PET imaging and drug discrimination learning. This will be useful, for example, in deciphering the function of the dorsal and ventral regions of the hippocampus, which appear to have different 5HT receptor-mediated effects [Gage and Springer, 1981] and should be further explored. Also, microinjection studies will be necessary to distinguish between the pre- and post-synaptic receptor effects of these compounds.

Primarily, the current study has helped to highlight some differences in the pharmacology of 5HT receptor-related drugs. This may prove to be important in the elucidation of the drugs' mechanisms of action and in clarifying the role of 5HT receptor subtypes in regulating central DA systems. In addition, these experiments are valuable in a therapeutic context since psychoactive compounds are clinically administered by systemic routes. The pharmacology of the 5HT receptors is complex, and once it is clearer, it will be possible to link the behavioral and neurochemical correlates of the serotonergic drugs.

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